DECLARATION

I, Akiko KOSEMURA, of HIRAKI & ASSOCIATES, do solemnly and sincerely declare as follows:

1. That I am well acquainted with the English and Japanese languages and am competent to translate from Japanese into English.

2. That I have executed, with the best of my ability, a true and correct translation into English of Japanese Patent Application No. 148242/2003 filed on May 26, 2003, a copy of which I attach herewith.

This 16th day of July, 2010

A. Kosemura

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[Title of Document] DESCRIPTION

[Title Of Invention] A NUCLEIC ACID CONSTRUCT CONTAINING A NUCLEIC ACID DERIVED FROM THE GENOME OF HEPATITIS C VIRUS (HCV) OF GENOTYPE 2a, AND A CELL HAVING SUCH NUCLEIC ACID CONSTRUCT INTRODUCED THEREIN

[CLAIMS]

[Claim 1]

A replicon RNA, comprising a nucleotide sequence containing at least the 5' untranslated region, the sequence encoding NS3 protein, NS4A protein, NS4B protein, NS5A protein and NS5B protein and the 3' untranslated region on the genomic RNA of hepatitis C virus of genotype 2a.

[Claim 2]

The replicon RNA of claim 1, further containing at least one selection marker gene or a reporter gene, and at least one IRES sequence.

[Claim 3]

A replicon RNA, comprising a nucleotide sequence containing the 5' untranslated region comprising the nucleotide sequence represented by SEQ ID NO: 9 or 10; at least one selection marker gene or a reporter gene; an IRES sequence; the sequence encoding NS3 protein, NS4A protein, NS4B protein, NS5A protein and NS5B protein on the genomic RNA of hepatitis C virus of genotype 2a; and the 3' untranslated region comprising the nucleotide sequence represented by SEQ ID NO: 11 or 12.

[Claim 4]

The replicon RNA of any one of claims 1 to 3, wherein the genomic RNA of hepatitis C virus of genotype 2a is an RNA comprising the nucleotide sequence represented by SEQ ID NO: 3 or 5.

[Claim 5]

A replicon RNA, comprising the following RNA (a) or (b):

(a) an RNA comprising the nucleotide sequence represented by SEQ ID NO: 1 or

2; and

(b) an RNA comprising a nucleotide sequence derived from the nucleotide sequence represented by SEQ ID NO: 1 or 2 by deletion, substitution or addition of 1 to 10 nucleotides, and being capable of autonomous replication.

[Claim 6]

A replicon-replicating cell, which is prepared by introducing the replicon RNA of any one of claims 1 to 5 into a cell.

[Claim 7]

The replicon-replicating cell of claim 6, wherein the cell is a eukaryotic cell.

[Claim 8]

The replicon-replicating cell of claim 7, wherein the eukaryotic cell is a human liver-derived cell.

[Claim 9]

The replicon-replicating cell of claim 7, wherein the eukaryotic cell is any one cell selected from the group consisting of an Huh7 cell.

[Claim 10]

The replicon RNA of any one of claims 1 to 5, which is for producing or evaluating a therapeutic agent or a diagnostic agent for treatment of hepatitis C virus infection.

[Claim 11]

The replicon-replicating cell of any one of claims 6 to 9, which is for producing or evaluating a therapeutic agent or a diagnostic agent for treatment of hepatitis C virus infection.

[Claim 12]

The replicon RNA of any one of claims 1 to 5, which is for producing a vaccine against hepatitis C virus infection.

[Claim 13]

The replicon-replicating cell of any one of claims 6 to 9, which is for producing a vaccine against hepatitis C virus infection.

[Claim 14]

A method of producing a replicon RNA of hepatitis C virus of genotype 2a, comprising extracting the replicon RNA from the replicon-replicating cell of any one of claims 6 to 9.

[Claim 15]

A method of producing a viral protein of hepatitis C virus of genotype 2a, comprising culturing the replicon-replicating cell of any one of claims 6 to 9, and obtaining the viral protein from the resulting culture product.

[Claim 16]

A method of screening for a substance promoting or suppressing the replication of hepatitis C virus, comprising culturing the replicon-replicating cell of any one of claims 6 to 9 in the presence of a test substance, and detecting the replication of a replicon RNA in the resulting culture product.

[Claim 17]

A method of introducing a mutation that increases the replication efficiency to the replicon RNA of hepatitis C virus of genotype 2a, comprising performing once or more the following: obtaining a replicated replicon RNA from the replicon-replicating cell of any one of claims 6 to 9, and introducing the thus obtained replicated replicon RNA into a cell that is different from the replicon-replicating cell so as to prepare a new replicon-replicating cell.

[Claim 18]

The method of claim 17, wherein the replication efficiency increases to become at least two times greater than that of the replicon RNA that is introduced at the beginning into the replicon-replicating cell.

[Claim 19]

A method of producing a replicon RNA of hepatitis C virus of genotype 2a having increased replication efficiency, comprising performing once or more the following: obtaining a replicated replicon RNA from the replicon-replicating cell of any one of claims 6 to 9, and introducing the thus obtained replicated replicon

RNA into a cell that is different from the replicon-replicating cell so as to prepare a new replicon-replicating cell; and obtaining a replicated replicon RNA from the finally obtained replicon-replicating cell.

[Claim 20]

A method of producing a replicon RNA of hepatitis C virus of genotype 2a having increased replication efficiency, comprising detecting a nucleotide mutation or an amino acid mutation between the replicon RNA that is produced so as to have an increased replication efficiency by the method of claim 19 and the replicon RNA that is introduced at the beginning into the replicon-replicating cell; and introducing the thus detected nucleotide mutation or amino acid mutation into a replicon RNA whose replication efficiency is to be increased.

[Claim 21]

A replicon RNA, comprising a nucleotide sequence derived from the nucleotide sequence represented by SEQ ID NO: 1 by at least one mutation selected from the group consisting of the following (a) to (h):

(a) a mutation from A to G at nucleotide site 7157;

(b) a mutation from C to U at nucleotide site 4955;

(c) a mutation from A to G at nucleotide site 4936;

(d) a mutation from A to G at nucleotide site 5000;

(e) a mutation from A to G at nucleotide site 7288;

(f) a mutation from G to U at nucleotide site 5901;

(g) a mutation from A to U at nucleotide site 6113; and

(h) a mutation from A to G at nucleotide site 2890.

[Detailed Description of Invention]

[0001]

[Technical Field of Invention]

The present invention relates to a replicon RNA of the hepatitis C virus of genotype 2a, a replicon-replicating cell wherein the replicon RNA is introduced, and a method of increasing the replication efficiency of the replicon RNA.

[0002]

[Conventional Art]

The hepatitis C virus (HCV) is a virus belonging to the family Flaviviridae. It has a single-stranded (+) strand sense RNA as its genome and is known to cause hepatitis C. Recent studies have revealed that Hepatitis C virus is classified into a number of types based on genotypes or serotypes. According to the phylogenetic analysis of Simmonds et al., using the nucleotide sequences of the HCV strains, which is currently a mainstream method of classifying HCV genotypes, HCV is classified into 6 genotypes: genotype 1a, genotype 1b, genotype 2a, genotype 2b, genotype 3a and genotype 3b (see Non Patent Literature 1). Each of these types is further classified into several subtypes. The nucleotide sequences of the full-length genomes of a several number of genotypes of HCV have been determined to date (see Patent Literature 1 and Non Patent Literatures 2-5).

[0003]

HCV causes chronic hepatitis by persistent infection. Currently, the main cause of chronic hepatitis observed worldwide is persistent HCV infection. Actually, around 50% of individuals with persistent infection develop chronic hepatitis. Chronic hepatitis in approximately 20% of these patients shifts to liver cirrhosis over the course of 10 to 20 years, and some of these patients further go on to advanced lethal pathological conditions such as hepatic cancer.

[0004]

Hepatitis C is currently treated mainly by a therapy using interferon-α or interferon-β, or a therapy using in combination interferon-α and ribavirin, the purine-nucleoside derivative. However, even when these therapies are performed, the therapeutic effects are observed in only approximately 60% of all the treated patients. When the therapies are ceased after the exertion of the effects, the disease recrudesces in more than half of the patients. The therapeutic effect of interferones is known to relate to HCV genotypes, and is said to be lower

against genotype 1b and higher against genotype 2a (see Non Patent Literature 6).

[0005]

It is an important goal to develop therapeutic agents or prophylactic agents effective against hepatitis C, the incidence rate of which is high in industrial countries, for which currently no causal treatment are present, and which finally bring about serious results. Hence, the development of HCV-specific chemotherapies and vaccine therapies are earnestly desired. A target for the development of an anti-HCV agent may be the suppression of HCV replication or the suppression of infection of cells with HCV.

[0006]

Until recently, propagation of HCV in a cell culture system and infecting cultured cells with HCV have been difficult. Moreover, a chimpanzee has been the only animal that can be infected with HCV and can be used in experiments, so that it has been difficult to carry out studies on the replication mechanism of HCV and the infection mechanism of HCV. However, recently, HCV subgenomic RNA replicons have been prepared as HCV-derived autonomously replicable RNA (see Patent Literature 2 and Non Patent Literatures 7-10), which enables the analysis of the replication mechanism of HCV using cultured cells. These HCV subgenomic RNA replicons are each prepared by substituting structural proteins existing downstream of HCV IRES in the 5' untranslated region of the HCV genomic RNA of genotype 1b with a neomycin resistance gene and EMCV IRES that has been ligated downstream of the resistance gene. It has been demonstrated that this RNA replicon is autonomously replicated in human hepatic cancer cells, Huh7 cells, when introduced into the Huh7 cells followed by culture in the presence of neomycin.

[0007]

However, regarding such intracellular RNA replication systems for HCV, only those using HCV genomic RNA of genotype 1b have been prepared so far. Since there has been a report that different genotypes of HCV differ also in viral

proteins encoded, it may be difficult to sufficiently elucidate the replication mechanism of HCV only by analyzing the subgenomic RNA replicons derived from HCV of genotype 1b. Furthermore, based on the fact that the therapeutic effects of interferons differ depending on the HCV genotypes, it may be particularly difficult to develop an anti-HCV agent having an effect on various types of HCV by the use of only an HCV replication system containing the subgenomic RNA replicon of HCV of genotype 1b.

[0008]

[Patent Literature 1] JP Patent Publication (Kokai) No. 2002-171978 A

[Patent Literature 2] JP Patent Publication (Kokai) No. 2001-17187 A

[Non Patent Literature 1] Simmonds, P. et al, Hepatology, (1994) 10, pp. 1321-1324

[Non Patent Literature 2] Choo et al., Science, (1989) 244, pp. 359-362

[Non Patent Literature 3] Kato et al., J. Med. Virol., (2001) 64(3) pp. 334-339

[Non Patent Literature 4] Okamoto, H et al, J. Gen. Virol., (1992) 73 pp. 673-679

[Non Patent Literature 5] Mori, S. et al, Biochem. Biophis. Res. Commun., (1992) 183, pp. 334-342

[Non Patent Literature 6] Yoshioka et al., Hepatology, (1992) 16(2): pp. 293-299

[Non Patent Literature 7] Lohmann et al., Science, (1999) 285, pp. 110-113

[Non Patent Literature 8] Blight et al., Science, (2000) 290, pp. 1972-1974

[Non Patent Literature 9] Friebe et al., J. Virol., (2001) 75(24): pp. 12047-12057

[Non Patent Literature 10] Ikeda et al., J. Virol., (2002) 76(6): pp. 2997-3006

[0009]

[Problem to be Solved by Invention]

An object of the present invention is to provide an HCV-derived replicon RNA of a HCV genotype for which replicon RNA has not yet been prepared.

[0010]

[Means for Solving the Problem]

As a result of intensive studies to achieve the above object, we have

succeeded in preparing the replicon RNA of HCV genotype 2a.

[0011]

That is, the present invention is as follows.

[1] A replicon RNA, comprising a nucleotide sequence containing at least the 5' untranslated region, the sequence encoding NS3 protein, NS4A protein, NS4B protein, NS5A protein and NS5B protein and the 3' untranslated region on the genomic RNA of hepatitis C virus of genotype 2a. Preferably, this replicon RNA further contains at least one selection marker gene or a reporter gene, and at least one IRES sequence.

[2] A replicon RNA, comprising a nucleotide sequence containing the 5' untranslated region comprising the sequence represented by either SEQ ID NO: 9 or 10; at least one selection marker gene or a reporter gene; an IRES sequence; the sequence encoding NS3 protein, NS4A protein, NS4B protein, NS5A protein and NS5B protein on the genomic RNA of hepatitis C virus of genotype 2a; and the 3' untranslated region comprising the nucleotide sequence represented by either SEQ ID NO: 11 or 12.

[3] The replicon RNA of [1] or [2] above, wherein the genomic RNA of hepatitis C virus of genotype 2a is an RNA comprising the nucleotide sequence represented by SEQ ID NO: 3 or 5.

[4] A replicon RNA, comprising the following RNA (a) or (b):

(a) an RNA comprising the nucleotide sequence represented by SEQ ID NO: 1 or 2; and

(b) an RNA comprising a nucleotide sequence derived from the nucleotide sequence represented by SEQ ID NO: 1 or 2 by deletion, substitution or addition of 1 to 10 nucleotides, and being capable of autonomous replication.

[5] A replicon-replicating cell, which is prepared by introducing the replicon RNA of any one of [1] to [4] above into a cell. For this replicon-replicating cell, a cell into which the replicon RNA is introduced is preferably a eukaryotic cell, more preferably a human liver-derived cell, and further more preferably an Huh7 cell.

[6] The replicon RNA of [1] to [4] above, which is for producing or evaluating a therapeutic agent or a diagnostic agent for treatment of hepatitis C virus infection.

[7] The replicon-replicating cell of [5] above, which is for producing or evaluating a therapeutic agent or a diagnostic agent for treatment of hepatitis C virus infection.

[8] The replicon RNA of [1] to [4] above, which is for producing a vaccine against hepatitis C virus infection.

[9] The replicon-replicating cell of [5] above, which is for producing a vaccine against hepatitis C virus infection.

[10] A method of producing a replicon RNA of hepatitis C virus of genotype 2a, comprising extracting the replicon RNA from the replicon-replicating cell of [5] above.

[11] A method of producing a viral protein of hepatitis C virus of genotype 2a, comprising culturing the replicon-replicating cell of [5] above, and obtaining the viral protein from the resulting culture product.

[12] A method of screening for a substance promoting or suppressing the replication of hepatitis C virus, comprising culturing the replicon-replicating cell of [5] above in the presence of a test substance, and detecting the replication of a replicon RNA in the resulting culture product.

[13] A method of introducing a mutation that increases the replication efficiency to the replicon RNA of hepatitis C virus of genotype 2a, comprising performing once or more the following: obtaining a replicated replicon RNA from the replicon-replicating cell of [5] above, and introducing the thus obtained replicated replicon RNA into a cell that is different from the replicon-replicating cell so as to prepare a new replicon-replicating cell. In this method, it is more preferred that the replication efficiency increases to become preferably at least two times greater than that of the replicon RNA that is introduced at the beginning into the replicon-replicating cell.

[14] A method of producing a replicon RNA of hepatitis C virus of genotype 2a

having increased replication efficiency, comprising performing once or more the following: obtaining a replicated replicon RNA from the replicon-replicating cell of [5] above, and introducing the thus obtained replicated replicon RNA into a cell that is different from the replicon-replicating cell so as to prepare a new replicon-replicating cell; and obtaining a replicated replicon RNA from the finally obtained replicon-replicating cell.

[15] A method of producing a replicon RNA of hepatitis C virus of genotype 2a having increased replication efficiency, comprising detecting a nucleotide mutation or an amino acid mutation between the replicon RNA that is produced so as to have an increased replication efficiency by the method of [14] above and the replicon RNA that is introduced at the beginning into the replicon-replicating cell; and introducing the thus detected nucleotide mutation or amino acid mutation into a replicon RNA whose replication efficiency is to be increased.

[16] A replicon RNA, comprising a nucleotide sequence derived from the nucleotide sequence represented by SEQ ID NO: 1 by at least one mutation selected from the group consisting of the following (a) to (h):

(a) a mutation from A to G at nucleotide site 7157;

(b) a mutation from C to U at nucleotide site 4955;

(c) a mutation from A to G at nucleotide site 4936;

(d) a mutation from A to G at nucleotide site 5000;

(e) a mutation from A to G at nucleotide site 7288;

(f) a mutation from G to U at nucleotide site 5901;

(g) a mutation from A to U at nucleotide site 6113; and

(h) a mutation from A to G at nucleotide site 2890.

[0012]

[Mode for Carrying out Invention]

The present invention is explained in detail as follows.

1. HCV-derived replicon RNA according to the present invention

The genome of hepatitis C virus (HCV) is a single-stranded (+) strand

RNA comprising approximately 9600 nucleotides. This genomic RNA comprises the 5' untranslated region (also denoted as 5' NTR or 5' UTR), a translated region composed of a structural region and a non-structural region and the 3' untranslated region (also denoted as 3' NTR or 3' UTR). HCV structural proteins are encoded in the structural region, and a plurality of non-structural proteins are encoded in the non-structural region.

[0013]

Such HCV structural proteins and non-structural proteins are generated through the translation into a continuous form thereof, a polyprotein, from the translated region, restricted degradation of the polyprotein by protease, and then the release of the structural proteins (Core, E1 and E2) and non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B), respectively. Among these structural proteins and non-structural proteins, that is, viral proteins of HCV, Core is a core protein, E1 and E2 are envelope proteins, and non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B) are proteins involved in virus's own replication. NS2 is known to have metalloprotease activity, and NS3 is known to have serine protease activity (at one-third of the N terminal side) and helicase activity (at two-thirds of the C-terminal side). Furthermore, NS4A is a cofactor for protease activity of NS3, and NS5B has been reported to have RNA-dependent RNA polymerase activity. Furthermore, the genome of HCV of genotype 2a has already been reported to have a similar gene structure (see Patent Literature 1).

[0014]

We have constructed RNA capable of autonomous replication using such HCV genome of genotype 2a. Specifically, the HCV-derived replicon RNA of the present invention is an RNA construct, which contains the whole or partial RNA of the HCV genome of genotype 2a and is capable of autonomous replication.

[0015]

In this specification, RNA that is prepared by altering the viral genome of HCV and is capable of autonomous replication is referred to as "replicon RNA" or

"RNA replicon." RNA that is artificially prepared from HCV of genotype 2a and is capable of autonomous replication is referred to as "replicon RNA derived from HCV of genotype 2a." In this specification, the HCV-derived replicon RNA is also referred to as an HCV-RNA replicon.

[0016]

In the present invention, "hepatitis C virus of genotype 2a" or "HCV of genotype 2a" means hepatitis C virus identified as genotype 2a according to the international classification of Simmonds et al. The "hepatitis C virus of genotype 2a" or the "HCV of genotype 2a" of the present invention encompasses not only a virus having naturally occurring HCV genomic RNA, but also a virus having genomic RNA prepared by artificially altering a naturally occurring HCV genomic sequence. Specific examples of HCV of genotype 2a include viruses of JFH-1 strain and the JCH-1 strain (see Patent Literature 1).

[0017]

Furthermore, "the genomic RNA of hepatitis C virus of genotype 2a" means RNA that comprises the single-stranded (+) strand sense RNA of hepatitis C virus of genotype 2a and has the nucleotide sequence throughout the entire region of its genome. The genomic RNA of hepatitis C virus of genotype 2a is preferably RNA comprising the nucleotide sequence represented by SEQ ID NO: 3 or 5, but is not limited thereto.

[0018]

In the specification of the present application, "5' untranslated region" (5'NTR or 5'UTR), "a sequence encoding NS3 protein, NS4A protein, NS4B protein, NS5A protein and NS5B protein," "a sequence encoding Core protein" (Core region or C region), "a sequence encoding E1 protein" (E1 region), "a sequence encoding E2 protein" (E2 region), "a sequence encoding N2 protein" (NS2 region), "a sequence encoding NS3 protein" (NS3 region), "a sequence encoding NS4A protein" (NS4A region), "a sequence encoding NS4B protein" (NS4B region), "a sequence encoding NS5A protein" (NS5A region), "a sequence

encoding NS5B protein" (NS5B region) and "3' untranslated region" (3' NTR or 3' UTR), and other specific regions or sites are determined based on the nucleotide sequence of SEQ ID NO: 3 of the full-length cDNA (JFH-1 clone) encoding the entire region of the genome of the JFH-1 strain, which is HCV of genotype 2a. The nucleotide sequence of SEQ ID NO: 3 can be obtained from the International DNA Data Bank (DDBJ/EMBL/GenBank) by referring to the accession No. AB047639. Specifically, when a particular HCV RNA sequence is aligned with the nucleotide sequence represented by SEQ ID NO: 3, a sequence to be aligned with nucleotides 1 to 340 on the nucleotide sequence represented by SEQ ID NO: 3 is "5' untranslated region" of the RNA, a sequence to be aligned with the nucleotides 3431 to 9442 on the same are a sequence encoding NS3 protein, NS4A protein, NS4B protein, NS5A protein and NS5B protein, a sequence to be aligned with the nucleotides 3431 to 5323 on the same is "a sequence encoding NS3 protein," a sequence to be aligned with the nucleotides 5324 to 5485 on the same is "a sequence encoding NS4A protein," a sequence to be aligned with the nucleotides 5486 to 6268 on the same is a sequence encoding NS4B protein," a sequence to be aligned with the nucleotides 6269 to 7666 on the same is "a sequence encoding NS5A protein," a sequence to be aligned with the nucleotides 7667 to 9442 on the same is "a sequence encoding NS5B protein," and a sequence to be aligned with the nucleotides 9443 to 9678 on the same is "3' untranslated region." Furthermore, in this case, gaps, additions, deletions, substitutions or the like may be present in the "aligned" sequences. Furthermore, the above "particular HCV" is not limited thereto, and includes the JFH-1 strain or JCH-1 strain, or viral strains that are derivatives thereof.

[0019]

One embodiment of the HCV RNA-replicon according to the present invention is a replicon RNA comprising a nucleotide sequence containing at least the 5' untranslated region, a sequence encoding NS3 protein, NS4A protein, NS4B protein, NS5A protein and NS5B protein, and the 3' untranslated region on the

genomic RNA of hepatitis C virus of genotype 2a. The replicon RNA may further contain at least one selection marker gene or one reporter gene, and at least one IRES sequence. Furthermore, this replicon RNA may also contain a sequence encoding a viral protein other than NS3, NS4A, NS4B, NS5A and NS5B proteins on the genomic RNA of hepatitis C virus of genotype 2a.

[0020]

Another preferred embodiment of HCV RNA-replicon according to the present invention is a replicon RNA comprising a nucleotide sequence containing the 5' untranslated region comprising the nucleotide sequence represented by SEQ ID NO: 9 or 10, at least one selection marker gene or reporter gene, the IRES sequence, a sequence encoding NS3 protein, NS4A protein, NS4B protein, NS5A protein and NS5B protein on the genomic RNA of hepatitis C virus of genotype 2a, and the 3' untranslated region comprising the nucleotide sequence represented by SEQ ID NO: 11 or 12. In this case the nucleotide sequences represented by SEQ ID NO: 9 and 10 are sequences of the 5' untranslated regions of rSGREP-JFH1 (SEQ ID NO: 1) and rSGREP-JCH1 (SEQ ID NO: 2), respectively, which are replicon RNAs according to the present invention. Furthermore, the nucleotide sequences represented by SEQ ID NO: 11 and 12 are sequences of the 3' untranslated regions of rSGREP-JFH1 (SEQ ID NO: 1) and rSGREP-JCH1 (SEQ ID NO: 2), respectively, which are replicon RNAs according to the present invention.

[0021]

A more preferred embodiment of HCV RNA-replicon according to the present invention is a replicon RNA comprised of an RNA comprising the nucleotide sequence represented by SEQ ID NO: 1 or 2. Furthermore, a replicon RNA comprising a nucleotide sequence derived from the nucleotide sequence represented by SEQ ID NO: 1 or 2 by deletion, substitution or addition of 1 to 50, 1 to 30, 1 to 10, 1 to 6, or 1 to several (2 to 5) nucleotides, and being capable of autonomous replication is also included in the scope of the present invention as a

preferred embodiment. In the present invention, "capable of autonomous replication" means that when replicon RNA is introduced into a cell, the replicon RNA allows its own full-length sequence to be replicated within the cell. For example, this ability of autonomous replication can be confirmed by transfecting replicon RNA into Huh7 cells, culturing the Huh7 cells, extracting RNA from the cells in the thus resulting culture product and conducting Northern blot hybridization for the extracted RNA using a probe that can specifically detect the transfected replicon RNA so as to detect the presence of the replicon RNA. However, examples of such a method are not limited thereto. Specific procedures for confirming the ability of autonomous replication can be conducted according to descriptions given in the Examples of this specification such as those for measuring the ability of colony formation, those for confirming the expression of HCV proteins or those for detecting replicon RNA.

[0022]

In the present invention, a "selection marker gene" means a gene that can provide a cell with selectivity such that only the cell expressing the gene is selected. A general example of a selection marker gene is an antibiotic resistance gene. In the present invention, preferred examples of a selection marker gene include a neomycin resistance gene, a thymidine kinase gene, a kanamycin resistance gene, a pyrithiamine resistance gene, an adenylyl transferase gene, a Zeocin resistance gene and a puromycin resistance gene. The neomycin resistance gene and the thymidine kinase gene are preferred, and the neomycin resistance gene is more preferred. However, the selection marker gene in the present invention is not limited to these genes.

[0023]

Furthermore in the present invention, a "reporter gene" means a marker gene encoding a gene product that is a marker for the expression of the gene. General examples of a reporter gene include structural genes of enzymes that catalyze light emitting reaction or color reaction. Preferred examples of the

reporter gene in the present invention include a transposon Tn9-derived chloramphenicol acetyltransferase gene, an Escherichia coli-derived β glucuronidase or β galactosidase gene, a luciferase gene, a green fluorescence protein gene, an aequorin gene from jellyfish, and a secreted placental alkaline phosphatase (SEAP) gene. However, the reporter gene in the present invention is not limited to these genes.

[0024]

Either only one or both of the above selection marker gene and reporter gene may be contained in replicon RNA.

[0025]

In the present invention, "IRES sequence" means an internal ribosome entry site that allows translation to be initiated by binding ribosomes within the inside of RNA. Preferred examples of IRES sequence in the present invention include, but are not limited to, EMCV IRES (the internal ribosome entry site of encephalomyocarditis virus), FMDV IRES and HCV IRES. EMCV IRES and HCV IRES are more preferred, and EMCV IRES is the most preferred sequence.

[0026]

The replicon RNA according to the present invention may further contain a sequence on the genomic RNA of another HCV strain or HCV of another genotype. For example, the replicon RNA may also contain a fragment of HCV genome of genotype 1b. Examples of another HCV strain include, but are not limited to, HCV-1, HCV-H, HC-J1, HCT-18, H77, DK-7, US11, S14, HCT23, HCV-Th, DR1, DR4, HCT27, S18, SW1, DK9, H90, TD-6E1, S9, HCV-BK, T10, DK1, HC-J4, HCV-J, HK3, HK8, HK5, HCV-G3, IND5, IND8, P10, D1, D3, SW2, T3, S45, SA10, US6, HCV-JK1, HCV-JK4, HCV-JK3, HCV-JK2, HCV-JT, HC-J2, HCV-T, HK4, HC-G9, Z1, Bi, S. I., Cho, J.M., HCV-J6, T4, T9, US10, HC-J5, T2, HC-J7, DK11, SW3, DK8, T8, HC-J8, S83, HK2, HC-J6, HC-J8, BEBE1, HCV-J6, HCV-J8, HD10-2, BR36-9, S52, S54, S2, BR33-1, HK10, DK12, HCV-TR, BA-1, BA-2, DK13, Z1, Z4, Z6, Z7, HK2, SA1, SA4, SA5, SA7, SA13, SA6, NZL1, SA30, EG-

13, HCV-K3a/650, ED43, EUH1480, EUHK2, Th580, VN235, VN405, VN004, JK049, JK046, JFH-1, JCH-1, JCH-2, JCH-3, JCH-4, JCH-5, JCH-6, J6CF and H77.

[0027]

The replicon RNA according to the present invention preferably has the 5' untranslated region on the genomic RNA of HCV of genotype 2a on the 5'-most side, and the 3 'untranslated region on the genomic RNA of HCV of genotype 2a on the 3'-most side. A selection marker gene or a reporter gene may be ligated upstream of the IRES sequence, or upstream or downstream of "the sequence encoding NS3 protein, NS4A protein, NS4B protein, NS5A protein and NS5B protein," or inserted in the middle of "the sequence encoding NS3 protein, NS4A protein, NS4B protein, NS5A protein and NS5B protein."

[0028]

The replicon RNA according to the present invention more preferably has the 5' untranslated region on the genomic RNA of HCV of genotype 2a on the 5'-most side, and a selection marker gene or a reporter gene, the IRES sequence and "the sequence encoding NS3 protein, NS4A protein, NS4B protein, NS5A protein and NS5B protein" downstream of the 5' untranslated region in this order, and the 3' untranslated region on the genomic RNA of HCV of genotype 2a on the 3'-most side.

[0029]

Examples of the replicon RNA according to the present invention may include an RNA containing any foreign gene to be expressed within a cell into which the replicon RNA is introduced, in addition to the sequences as described above. A foreign gene may also be ligated downstream of the 5' untranslated region, or ligated upstream or downstream of a selection marker gene or a reporter gene, or ligated upstream or downstream of "the sequence encoding NS3 protein, NS4A protein, NS4B protein, NS5A protein and NS5B protein," or may be inserted in the middle of "the sequence encoding NS3 protein, NS4A protein, NS4B protein,

NS5A protein and NS5B protein." A replicon RNA containing a foreign gene can express a protein encoded by the foreign gene when it is translated within a cell into which the RNA is introduced. Thus, the replicon RNA containing a foreign gene can be appropriately used also for gene therapy or the like, the purpose of which is to generate a particular gene product within a cell.

[0030]

The replicon RNA according to the present invention may further contain a ribozyme. A ribozyme is inserted to ligate a selection marker gene, a reporter gene or a foreign gene on the 5' side in the replicon RNA to those located on the 3' side thereof including the IRES sequence and "the sequence encoding NS3 protein, NS4A protein, NS4B protein, NS5A protein and NS5B protein," so that it enables cleavage and separation of the two by the self-cleavage activity of the ribozyme.

[0031]

In the replicon RNA according to the present invention, the above described selection marker gene, reporter gene, sequences encoding viral proteins on the genomic RNA of hepatitis C virus of genotype 2a, sequences encoding viral proteins of HCV of a genotype other than genotype 2a, a foreign gene or the like are ligated so that they are translated from the replicon RNA in the correct reading frame. Among these sequences, the protein-coding sequences may be ligated to each other via a protease cleavage site and the like, so that after the proteins are expressed as a fusion protein with the polyprotein that is translated from "the sequence encoding NS3 protein, NS4A protein, NS4B protein, NS5A protein and NS5B protein" of hepatitis C virus of genotype 2a, the fusion protein is separated by protease into each protein.

[0032]

2. Preparation of replicon RNA according to the present invention

The HCV RNA-replicon according to the present invention can be prepared using any genetic engineering techniques known by persons skilled in the art. The HCV RNA-replicon can be prepared by, for example, the following method,

but the method of preparation is not limited thereto.

[0033]

First, DNA corresponding to the entire region of the genomic RNA of hepatitis C virus of genotype 2a is ligated downstream of an RNA promoter according to a standard procedure so as to prepare a DNA clone. As used herein, "DNA corresponding to RNA" means a DNA having a nucleotide sequence derived from the nucleotide sequence of the RNA by substituting U (uracil) with T (thymine). The above RNA promoter is preferably an RNA promoter contained in a plasmid clone. An example of an RNA promoter is not limited, but T7 RNA promoter is particularly preferred.

[0034]

Next, for the thus prepared DNA clone, for example, the structural region (Core sequence, E1 sequence and E2 sequence) located downstream of the 5' untranslated region and the sequence encoding NS2 protein are substituted with a DNA fragment containing a selection marker gene or a reporter gene and the IRES sequence ligated downstream thereof. In this substitution, portions other than the structural region, such as a fragment on the 3' terminal side of the 5' untranslated region or a part of the sequence encoding NS3 protein may be substituted with a sequence derived from HCV of another genotype.

[0035]

Subsequently, using the DNA clone after the substitution as a template, RNA is synthesized using RNA polymerase. RNA synthesis can be initiated by a standard procedure from the 5' untranslated region and the IRES sequence. When a template DNA is a plasmid clone, the above DNA region ligated downstream of an RNA promoter is excised by a restriction enzyme from the plasmid clone, and then RNA can be synthesized using the DNA fragment as a template. In addition, preferably the 3' terminus of RNA to be synthesized agrees with the 3' untranslated region of the viral genomic RNA, and no other sequences are added or deleted. The thus synthesized RNA is the replicon RNA according to the present invention.

[0036]

3. Preparation of replicon-replicating cells into which replicon RNA from HCV of genotype 2a is introduced

The replicon RNA that is prepared as described above is introduced into cells in which the replicon RNA should be replicated, so that cells wherein the replicon RNA is continuously replicated can be obtained. In this specification, a cell wherein replicon RNA is continuously replicated is referred to as a "replicon-replicating cell."

[0037]

As a cell into which replicon RNA is introduced, any cell can be used, as long as it can be subcultured. Such a cell is preferably a eukaryotic cell, more preferably a human liver-derived cell, and further preferably Huh7 cells. As these cells, commercially available cells may be utilized, these cells may be obtained from cell depositories, or cell lines established from any cells (e.g., cancer cells or stem cells) may also be used.

[0038]

Introduction of replicon RNA into cells can be performed using any technique known by persons skilled in the art. Examples of such an introduction method include electroporation, a particle gun method, a lipofection method, a calcium phosphate method, a microinjection method and a DEAE sepharose method. The method using electroporation is particularly preferred.

[0039]

A replicon RNA of interest may be introduced alone, or may be introduced after it is mixed with other nucleic acids. To vary the quantity of replicon RNA while keeping RNA quantity to be introduced at a certain level, the replicon RNA of interest is mixed with total cellular RNA extracted from cells into which the RNA is introduced, and then the mixture is used for introduction into cells. The quantity of replicon RNA to be used for introduction into cells may be determined depending on the introduction method employed, and is preferably between 1

picogram and 100 micrograms, and more preferably between 10 picograms and 10 micrograms.

[0040]

When replicon RNA containing a selection marker gene or a reporter gene is used for introduction into cells, cells wherein the replicon RNA is introduced and continuously replicated can be selected utilizing the expression of the selection marker gene or the reporter gene. Specifically, for example, such cells into which replicon RNA has been introduced may be cultured in media whereby the cells can be selected by the expression of the selection marker gene or the reporter gene. As an example, when replicon RNA contains a neomycin resistance gene as a selection marker gene, cells into which replicon RNA has been intracellularly introduced are seeded into a culture dish. After 16 to 24 hours of culture, G418 (neomycin) is added to the culture dish at a concentration of 0.05 milligrams/milliliter to 3.0 milligrams/milliliter. The cells are continuously cultured for preferably 10 days to 40 days and more preferably 14 days to 28 days after seeding, while exchanging the culture solution twice a week. Next, surviving cells are stained with crystal violet, so that cells into which the replicon RNA has been introduced and is being continuously replicated can be selected as formed colonies.

[0041]

Cloned cells can be obtained from the formed colonies by cloning surviving cells by a standard procedure, and then continuing the culture of the cells. The thus obtained cell clone wherein the replicon RNA of interest is continuously replicated is referred to as "a replicon-replicating cell clone" in this specification.

[0042]

Regarding the established cell clone, detection of a replicon RNA that has been replicated from the introduced replicon RNA in the cell clone, confirmation of the presence or the absence of the incorporation of a selection marker gene or a

reporter gene in the introduced replicon RNA into a host genomic DNA, and confirmation of the expression of an HCV protein are preferably carried out to confirm the fact that a replicon RNA of interest is actually and continuously replicated.

[0043]

A replicon RNA that has been replicated from the introduced replicon RNA in the cell clone (in this specification, hereinafter conveniently referred to as "replicated replicon RNA") may be detected according to any RNA detection method known by persons skilled in the art. For example, detection can be performed by conducting the Northern hybridization method for total RNA extracted from the cell clone using as a probe a DNA fragment specific to the introduced replicon RNA.

[0044]

Furthermore, the presence or the absence of the incorporation of a selection marker gene or a reporter gene in the introduced replicon RNA into a host genomic DNA can be confirmed by, for example, performing PCR for the host genomic DNA extracted from the cell clone to amplify at least a part of the selection marker gene or the reporter gene, and then confirming the presence or the absence of the amplified product. However, examples of relevant methods are not limited thereto. A cell clone for which the amplified product is confirmed is considered to have a selection marker gene or a reporter gene incorporated in the host genome. Thus, regarding the cell clone, the replicon RNA itself may not be continuously replicated within the cell. In this case, whether or not the replicon RNA is continuously replicated can be confirmed by conducting an experiment to confirm the expression of an HCV protein, as described below.

[0045]

The expression of an HCV protein can be confirmed by, for example, causing an antibody against an HCV protein to be expressed from the introduced replicon RNA and to react with a protein extracted from a cell clone. This

method can be conducted by any protein detection method known by persons skilled in the art. Specifically, for example, a protein sample extracted from the cell clone is blotted onto a nitrocellulose membrane, with which an anti-HCV protein antibody (e.g., an anti-NS3-specific antibody or an antiserum collected from a hepatitis C patient) is reacted, and then the anti-HCV protein antibody is detected. If the HCV protein is detected among proteins extracted from the cell clone, it can be concluded that this cell clone continuously replicate HCV-derived replicon RNA to express the HCV protein.

[0046]

As described above, cell clones confirmed to continuously replicate a replicon RNA of interest (replicon-replicating cell clones) can be obtained. Furthermore in the present invention, replicon RNA can be obtained by any method known by persons skilled in the art, for example, by extracting RNA from the replicon-replicating cell, and then separating replicon RNA from the RNA by an electrophoresis method. The present invention also relates to such a method of producing replicon RNA. Moreover, preferably, the replicon-replicating cell according to the present invention can be used for producing HCV proteins. Persons skilled in the art can obtain HCV proteins from the replicon-replicating cells according to any standard method. Specifically, for example, a viral protein of hepatitis C virus of genotype 2a can be produced by culturing replicon-replicating cells, collecting proteins from the resulting culture product (including cultured cells and culture media) by a standard procedure, and then selectively obtaining viral proteins from the proteins by detection or the like using an anti-HCV protein antibody.

[0047]

Moreover, when the replicon-replicating cell according to the present invention continuously replicates replicon RNA containing a foreign gene, a protein encoded by the foreign gene can be obtained by the expression thereof using the replicon-replicating cell. Specifically, for example, the protein encoded

by a foreign gene can be obtained by culturing replicon-replicating cells, collecting proteins from the resulting culture product (including cultured cells and culture media) by a standard procedure, and then selectively obtaining the protein from among the proteins by detection or the like using an antibody against the protein of interest.

[0048]

4. Introduction of mutation that increases replication efficiency into replicon RNA from HCV of genotype 2a

Mutation producing enhancement of replication efficiency frequently takes place in the replicon RNA that is replicated or generated in the replicon-replicating cell (replicated replicon RNA) according to the present invention. Such a mutation may be an adaptive mutation.

[0049]

Utilizing this fact, introduction of a mutation enhancing replication efficiency into the replicon RNA according to the present invention can be promoted in the present invention.

[0050]

Specifically, the step comprising obtaining a first replicated replicon RNA by extraction or the like from a first replicon-replicating cell (preferably, a replicon-replicating cell, wherein the replicon RNA according to the present invention has been introduced), and then re-introducing the first replicated replicon RNA into another cell to prepare a second replicon-replicating cell is performed repeatedly once or more, preferably 1 to 10 times, more preferably 1 to 5 times, and further preferably 1 to 2 times, so that the mutation increasing replication efficiency can be introduced at a high frequency into the replicon RNA within the replicon-replicating cells.

[0051]

As a cell into which a replicated replicon RNA is re-introduced, any cell can be used. Such a cell is preferably derived from a biological species that is

the same as that of a cell wherein replicon RNA is introduced at the beginning, more preferably derived from the same tissue derived from the same biological species as that of a cell wherein replicon RNA is introduced at the beginning, and further preferably of a cell line that is the same as that for a cell wherein replicon RNA is introduced at the beginning.

[0052]

Therefore in the present invention, using the above method, replicon RNA wherein the mutation increasing replication efficiency is introduced can be produced. Specifically, the step comprising obtaining a first replicated replicon RNA by extraction or the like from a first replicon-replicating cell (preferably, a replicon-replicating cell, into which the replicon RNA according to the present invention has been introduced), and then re-introducing the first replicated replicon RNA into another cell so as to prepare a second replicon-replicating cell is performed repeatedly once or more, preferably 1 to 10 times, more preferably 1 to 5 times, and further preferably 1 to 2 times. Subsequently, the replicated replicon RNA is obtained by extraction or the like from the replicon-replicating cell finally obtained at the end of the repeated steps, so that replicon RNA with increased replication efficiency can be produced.

[0053]

In the present invention, the replication efficiency of a replicon RNA can be increased at least 2 times, preferably 10 to 100 times, and more preferably 100 to 10000 times by the above method.

[0054]

Regarding the replicon RNA that is produced by such a method so as to have increased replication efficiency, the nucleotide sequence is preferably determined by a known method, for example, by obtaining cDNA by reverse transcription PCR and subjecting such cDNA to sequencing. Furthermore, the thus determined nucleotide sequence or the amino acid sequence encoded by the nucleotide sequence is compared with the nucleotide sequence of replicon RNA

that had been introduced at the beginning into cells, so that adaptive mutation can be identified. As adaptive mutation increasing replication efficiency, in particular, nonsynonymous substitution that mutates an amino acid in a viral protein encoded by replicon RNA is preferred.

[0055]

The present invention also provides a method whereby the replicon RNA of hepatitis C virus of genotype 2a having increased replication efficiency can be produced by introducing the thus identified adaptive mutation into replicon RNA, the replication efficiency of which is to be increased, by a standard procedure.

[0056]

The replicon RNA that is produced as described above so as to have increased replication efficiency can be used for producing replicon RNA in large quantity within cells that have been used for the method.

[0057]

The replication efficiency of the replicon RNA according to the present invention can be determined by a method known by persons skilled in the art. For example, it can be determined according to the following method. Replicon RNAs are transfected in quantities of 0.0001, 0.0003, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3 and 1.0 micrograms, respectively, into Huh7 cells, selective culture with G418 is performed for 21 days in a method similar to the above experimental techniques, and then the number of colonies formed (number of colonies) is counted. The quantity of RNA introduced is compared with the number of colonies formed to determine the range of the quantity of the replicon RNA introduced, within which colony formation increases in a quantity-dependent manner. The number of colonies formed within the range is divided by the quantity of RNA introduced, and the resulting value is regarded as the colony forming activity per microgram. This equation is as follows.

Colony forming activity [(Colony Forming Unit, or CFU)/microgram] = Number of colonies formed [colony] / quantity of RNA introduced [microgram]

[0058]

The thus calculated colony forming activity is regarded as a value representing the replication efficiency of replicon RNA introduced. Specifically, the higher the colony forming activity, the higher the replication efficiency of the replicon RNA.

[0059]

In addition, the replication efficiency of replicon RNA can also be shown via a colony-forming ability that is represented by the number of copies of the replicon RNA introduced per formed colony. That is, in this case, the ability can be calculated according to the following equation.

Colony forming ability = number of copies of replicon RNA introduced [copy] / number of formed colonies [colony]

[0060]

5. Other embodiments of the present invention

The replicon RNA-replicating cell according to the present invention can also be used as a test system for, for example, screening for a substance that promotes or suppresses the replication of hepatitis C virus. Specifically, for example, replicon replicating cells are cultured in the presence of a test substance, replication of the replicon RNA in the resulting culture product is detected, and then whether or not the test substance promotes or suppresses the replication of the replicon RNA is determined, so that a substance that promotes or suppresses the replication of hepatitis C virus can be screened for. In this case, detection of the replication of the replicon RNA in the resulting culture product may be conducted by detecting the quantity of, or the presence or the absence of, the replicon RNA in the RNAs extracted from the replicon RNA-replicating cell, or by detecting the quantity of, or the presence or the absence of, HCV protein contained in the proteins in the culture product or in the replicon RNA-replicating cells contained in the culture product.

[0061]

Such a test cell system using the replicon RNA-replicating cells according to the present invention may be aimed at producing or evaluating a therapeutic agent or a diagnostic agent for treating hepatitis C virus infection. Specific examples of such purposes include the following examples.

[0062]

(1) Search for a substance suppressing the proliferation of HCV of genotype 2a. Examples of such substance include organic chemicals directly or indirectly affecting the proliferation of HCV of genotype 2a, and antisense oligonucleotides directly or indirectly affecting the proliferation of HCV or the translation of HCV proteins by hybridizing to a target sequence in the HCV genome of genotype 2a or a complementary strand thereof.

(2) Evaluation of various substances having antiviral action in cell culture. Examples of the various substances include substances obtained through rational drug design or high throughput screening (e.g., an isolated and purified enzyme) and the like.

(3) Identification of a new target for attack for treating patients infected with HCV of genotype 2a. To identify a host cellular protein that plays an important role in proliferation of HCV virus, for example, the replicon-replicating cell according to the present invention can be used.

(4) Evaluation of the ability of HCV virus to acquire resistance against a drug or the like and identification of mutation concerning such resistance.

[0063]

The replicon RNA or replicon RNA-replicating cells according to the present invention may be aimed at the following purposes.

(5) Production of a viral protein as an antigen that can be used for developing, producing and evaluating a diagnostic agent or a therapeutic agent for hepatitis C virus infection.

(6) Viral genome replication system for producing HCV virus or virus-like particles that can be used for developing, producing and evaluating a diagnostic

agent or a therapeutic agent for hepatitis C virus infection.

(7) Production of a vaccine antigen that can be used as a vaccine against HCV of genotype 2a.

(8) Production of hepatic cell-directed genetic vector that is used after the incorporation of a foreign gene therein for gene therapy.

[0064]

[Examples]

The present invention will be described more specifically based on the following examples and drawings. However, the technical scope of the present invention is not limited by these examples.

[0065]

[Example 1] Preparation of replicon RNA

(A) Construction of expression vector

DNA corresponding to the entire region of viral genome of hepatitis C virus JFH-1 strain (genotype 2a) that had been separated from patients with fulminant hepatic failure was obtained from a JFH-1 clone containing the full-length genomic cDNA of the virus strain. The DNA was inserted downstream of T7 RNA promoter sequence that had been inserted in pUC19 plasmid. The thus constructed plasmid DNA is hereinafter referred to as pJFH1. Similarly, DNA corresponding to the entire region of viral genome of hepatitis C virus JCH-1 strain (genotype 2a) that had been separated from patients with chronic hepatitis was obtained from a JCH-1 clone containing the full-length genomic cDNA of the virus strain. The DNA was inserted downstream of the T7 RNA promoter sequence that had been inserted in pUC19 plasmid. The thus constructed plasmid DNA is hereinafter referred to as pJCH1. In addition, the preparation of the above JFH1 clone and JCH-1 clone is described in Patent Literature 1 and Non Patent Literature 3. Moreover, the nucleotide sequence of the full-length cDNA of JFH-1 clone was registered at the International DNA Data Bank (DDBJ/EMBL/GenBank) under accession No. AB047639, and the nucleotide

sequence of the full-length cDNA of the JCH-1 clone under accession No. AB047640.

[0066]

The structures of the thus constructed plasmid DNA pJFH1 and pJCH1 are shown in the upper section of Fig. 1. "T7" represents T7 RNA promoter, and "G" represents dGTP inserted upstream of the 5' end of the inserted JFH-1- or JCH-1-derived DNA and downstream of the 3' end of T7 RNA promoter sequence. A region from "5' NTR" to "3' NTR" is DNA corresponding to the entire genomic region of hepatitis C virus.

[0067]

Next, the structural regions and a part of the non-structural regions of plasmid DNA pJFH1 and pJCH1 were substituted with a neomycin resistance gene (neo; also referred to as a neomycin phosphotransferase gene) and EMCV-IRES (internal ribosome entry site of encephalomyocarditis virus), thereby constructing plasmid DNA pSGREP-JFH1 and pSGREP-JCH1, respectively (lower section of Fig. 1). This construction procedure was conducted according to a previous report (Non Patent Literature 7). Specifically, plasmid pJFH1 and pJCH1 were cleaved with restriction enzymes Age I and Cla I, and between the Age I and Cla I restriction sites, the following fragments were inserted to be ligated; a fragment was prepared by binding of a sequence ranging from 5' NTR to Core region derived from pJFH-1 with the neomycin resistance gene derived from pRSV5NEO by PCR amplification and then cleaving it with restriction enzymes Age I and Pme I, and, a fragment was prepared by binding of sequences ranging from EMCV IRES to NS3 region by PCR amplification and then cleaving it with restriction enzymes Pme I and Cla I.

[0068]

Moreover, a mutation that mutates an amino acid motif GDD to GND, corresponding to the active center of RNA polymerase encoded by the NS5B region, was introduced into the NS5B region in pSGREP-JFH1, thereby preparing

a mutant plasmid clone pSGREP-JFH1/GND.

[0069]

Moreover, a mutation that results in the deletion of a sequence of 10 continuous amino acids containing an amino acid motif GDD corresponding to the active center of RNA polymerase encoded by the NS5B region was introduced into the NS5B region in pSGREP-JFH1, thereby preparing a mutant plasmid clone pSGREP-JFH1/dGDD.

[0070]

The above-prepared mutant clones pSGREP-JFH1/GND and pSGREP-JFH1/dGDD cannot express active NS5B protein, which is required for the replication of replicon RNA, because the amino acid sequence of the active site of NS5B protein encoded by these clones has mutated.

[0071]

(B) Preparation of replicon RNA

To prepare template DNA for use in synthesis of replicon RNA, the above-constructed expression vectors pSGREP-JFH1, pSGREP-JCH1, pSGREP-JFH1/GND and pSGREP-JFH1/dGDD were each cleaved with a restriction enzyme Xba I.

[0072]

Subsequently, 10 to 20 μg each of these Xba I-cleaved fragments was contained in 50 μl of a reaction solution, and then further treated by 30 minutes of incubation at 30°C with 20 U of Mung Bean Nuclease. Mung Bean Nuclease is an enzyme catalyzing a reaction for selectively degrading a single-stranded portion of double-stranded DNA. Generally, when RNA synthesis is performed using directly the above Xba I-cleaved fragment as a template, a replicon RNA having four nucleotides of CUGA, a part of the recognition sequence of Xba I, excessively added to the 3' terminus would be synthesized. Hence, in this example, Xba I-cleaved fragments were treated with Mung Bean Nuclease, so as to remove the four nucleotides of CUGA from the fragments. The solutions

containing Xba I-cleaved fragments, which had been treated with Mung Bean Nuclease, were treated to remove proteins according to a general method, so that Xba I-cleaved fragments, from which the four nucleotides of CUGA had been removed, were purified and used as template DNAs.

[0073]

Next, from the template DNA, RNA was synthesized in vitro using T7 RNA polymerase. For this RNA synthesis, MEGAscript from Ambion, Inc. was used. Reaction was carried out using 20 μl of a reaction solution containing 0.5 to 1.0 micrograms of the template DNA according to the instructions of the manufacturer.

[0074]

After completion of RNA synthesis, DNase (2 U) was added to the reaction solution to conduct reaction at 37°C for 15 minutes. RNA extraction using acidic phenol was further performed to remove the template DNA. RNAs (replicon RNAs) synthesized in this manner from the above template DNAs derived from pSGREP-JFH1, pSGREP-JCH1, pSGREP-JFH1/GND and pSGREP-JFH1/dGDD were respectively named rSGREP-JFH1, rSGREP-JCH1, rSGREP-JFH1/GND and rSGREP-JFH1/dGDD. Regarding the nucleotide sequences of these replicon RNAs, the nucleotide sequence of rSGREP-JFH1 is shown in SEQ ID NO: 1 and Fig. 2, that of rSGREP-JCH1 is shown in SEQ ID NO: 2 and Fig. 3, that of rSGREP-JFH1/GND is shown in SEQ ID NO: 7, and that of rSGREP-JFH1/dGDD is shown in SEQ ID NO: 8.

[0075]

[Example 2] Establishment of replicon-replicating cell clone

(C) Transfection of replicon RNA, determination of colony-forming ability of transfected cells and establishment of cell clones

Each of the above-synthesized replicon RNAs (rSGREP-JFH1, rSGREP-JCH1, rSGREP-JFH1/GND and rSGREP-JFH1/dGDD) was mixed in different quantities with total cellular RNA extracted from Huh7 cells so as to have a total

RNA quantity of 10 μg. Subsequently, the mixed RNA was introduced into Huh7 cells by the electroporation method. The Huh7 cells subjected to the electroporation treatment were seeded into culture dishes, and then cultured for 16 hours to 24 hours. G418 (neomycin) was then added to the culture dishes at different concentrations. Thereafter, culture was continued while exchanging the culture solutions twice a week. After 21 days of culture following seeding, surviving cells were stained with crystal violet. The number of stained colonies was counted, and then the number of colonies obtained per μg of the transfected replicon RNA was calculated.

[0076]

For rSGREP-JFH1 or rSGREP-JCH1-transfected cells, for which colony formation had been observed, colonies of the surviving cells were further cloned from the above culture dishes after 21 days of culture, and were continuously cultured. By such cloning of colonies, several strains of cell clones could be established.

[0077]

For the established cell clones, detection of the replicated replicon RNA, confirmation of the presence or the absence of the incorporation of the neomycin resistance gene into the host genomic DNA, and confirmation of the expression of HCV proteins were performed as described later, in Example 4. Cell clones for which the replication of the replicon had been confirmed in the cells were regarded as replicon-replicating cell clones.

[0078]

(D) Colony-forming ability in each transfected cell

As a result of the above transfection, for rSGREP-JFH1-transfected Huh7 cells, the colony-forming ability per μg of the transfected replicon RNA was 94700 CFU (Colony Forming Unit)/μg·RNA when G418 concentration was 1.0 mg/ml (the left column in Fig. 4). In contrast, colony formation was not observed in the Huh7 cells, into which rSGREP-JFH1/dGDD and rSGREP-

JFH1/GND had each been transfected (the central column and the right column in Fig. 4). This suggests that the colony-forming ability confirmed for the Huh7 cells, into which rSGREP-JFH1 replicon RNA had been transfected, depends on the activity of NS5B (RNA polymerase) expressed by rSGREP-JFH1. Specifically, it was considered that in cells that had formed colonies, rSGREP-JFH1 replicon RNA autonomously replicated due to the action of NS5B expressed by rSGREP-JFH1, and the neomycin resistance gene was continuously expressed to maintain G418 resistance, so that cell growth was enabled.

[0079]

On the other hand, in the Huh7 cells, into which rSGREP-JCH1 had been transfected, no colony formation was observed in the case of 1 to 0.5 mg/ml G418 concentrations (Fig. 5). When G418 concentration was lowered to 0.25 mg/ml, colony formation was observed in the Huh7 cells, into which rSGREP-JCH1 had been transfected as well.

[0080]

Furthermore, Xba I-cleaved fragment of the expression vector pSGREP-JFH1 obtained in (B) above was used as a template DNA for RNA synthesis without treating the fragment with Mung Bean Nuclease, so as to synthesize replicon RNA. This replicon RNA was transfected to Huh7 cells in a manner similar to that in (C) above. The replicon RNA that had been prepared without performing Mung Bean Nuclease treatment had the four nucleotides of CUGA excessively added to the 3' terminus.

[0081]

As a result, the colony-forming ability of the Huh7 cells, into which the replicon RNA prepared without treatment with Mung Bean Nuclease had been transfected, decreased to 512 CFU/μg·RNA (the left side in Fig. 6). This result revealed that the sequence on the 3' terminus of the replicon RNA affects the colony-forming ability of the transfected cells.

[0082]

[Example 3]

(E) Re-transfection of replicated replicon RNA derived from replicon-replicating cells

From the replicon-replicating cell clones that had been established by transfection of rSGREP-JFH1 into Huh7 cells according to descriptions of Example 2, total RNA was extracted by a standard procedure. The number of copies of the replicated replicon RNA contained in the cellular RNA was determined by Northern blot analysis and a quantitative RT-PCR method.

[0083]

Northern blot analysis was performed according to the description in Molecular Cloning, A laboratory Manual, 2nd edition, J. Sambrook, E. F. Fritsch, T. Maniatis, Cold Spring Harbor Laboratory Press (1989). Specifically, RNA extracted from the cells was subjected to denaturing agarose electrophoresis. After electrophoresis, the RNA was transferred onto a positively charged nylon membrane. The ^{32}P-labeled DNA or RNA probe prepared from pSGREP-JFH1 was hybridized to the RNA transferred to the membrane as described above. Next the membrane was washed, and then exposed to a film, so as to detect a replicon-specific RNA band.

[0084]

Detection of the replicon RNA by quantitative RT-PCR was conducted by detecting the 5' untranslated region RNA within HCV RNA according to Takeuchi T, Katsume A, Tanaka T, Abe A, Inoue K, Tsukiyama-Kohara K, Kawaguchi R, Tanaka S and Kohara M., Real-time detection system for quantification of Hepatitis C virus genome, Gastroenterology 116: 636-642 (1999). Specifically, the replicon RNA contained in RNA extracted from the cells was amplified by PCR using synthetic primers: R6-130-S17, 5'-CGGGAGAGCCATAGTGG-3' (SEQ ID NO: 13) and R6-290-R19, 5'-AGTACCACAAGGCCTTTCG-3' (SEQ ID NO: 14); TaqMan Probe; R6-148-S21FT, 5'-CTGCGGAACCGGTGAGTACAC-3' (SEQ ID NO: 15) and an EZ rTth RNA PCR kit, and then detected using an ABI Prism

7700 sequence detector system.

[0085]

Next, aliquots of total cellular RNAs extracted from clone 6 (among the above-mentioned replicon-replicating cell clones) and pool clones (prepared by collecting replicon-replicating cells that had formed colonies from whole one dish and culturing them) were each introduced into another Huh7 cells by retransfection. Total cellular RNA used for the transfection was prepared to contain $1x10^7$ copies of replicon RNA based on the number of copies of the above-determined replicon RNA. Transfection was performed as described in (C) above, and then selective culture was performed under G418 concentration conditions of 1 mg/ml. Thus, the colony formation of the replicon-replicating cells was observed (Fig. 7). The colony-forming ability in this case was 1 colony or more per $1x10^6$ copies of the replicon RNA used for transfection, when it was calculated from the number of colonies obtained.

[0086]

On the other hand, the number of copies of in vitro synthetic RNA that had been synthesized in vitro using pSGREP-JFH1 as a template and T7 RNA polymerase was approximately $2x10^{11}$ copies/μg·RNA, when calculated based on the weight and the length of the RNA. The colony-forming ability in the case of using the in vitro synthetic RNA for transfection in a manner similar to the above method was 1 colony per $5x10^7$ copies. These results revealed that when RNA derived from cells extracted from replicon-replicating cells and in vitro synthetic RNA were each transfected to Huh7 cells as replicon RNA in the same number of copies, the use of the replicon RNA replicated within Huh7 cells resulted in colony-forming ability approximately 50 times higher than that of the in vitro synthetic RNA.

[0087]

[Example 4]

(F) Detection of replicon RNA

According to (E) above, cell clones [clones Nos. 1 to 11] were established by retransfection of total RNA that had been obtained from the replicon-replicating cell clone established by transfection of rSGREP-JFH1 to Huh7 cells to another Huh7 cells. From the established cell clones and pool clones (prepared by collecting cell clones that had formed colonies from whole one dish and then culturing them), respectively, total RNAs were extracted by an acidic phenol extraction method. Subsequently the total RNAs were analyzed by the Northern blot method using a pSGREP-JFH1-specific probe as a probe. As control, total RNA extracted similarly from untransfected Huh7 cells (in Fig. 8, denoted as "Huh7"), a sample prepared by adding 10^7 copies of replicon RNA synthesized in vitro to the total RNA extracted from Huh7 cells (in Fig. 8, denoted as "10^7"), and a sample (in Fig. 8, denoted as "10^8") prepared by adding 10^8 copies of replicon RNA synthesized in vitro to the total RNA extracted from Huh7 cells, were used. In Fig. 8, 1 to 11 represent cell clone Numbers.

[0088]

As a result, RNA of approximately the same size as that of rSGREP-JFH1 was detected using a pSGREP-JFH1-specific probe (Fig. 8). Thus, it was confirmed that the replicon RNA from rSGREP-JFH1 that had been transfected at the beginning replicated and proliferated within the cell clones. In addition, it was shown that the cell clones differed from each other in the quantity of the replicated replicon RNA. In Fig. 8, for example, clones 2, 6, 9 and 10 contained high quantities of the replicated replicon RNA, and clones 4, 8 and 11 contained low quantities of the replicated replicon RNA.

[0089]

(G) Confirmation of the presence or the absence of the incorporation of a neomycin resistance gene into genomic DNA

For the cell clones that had been obtained by retransfection of replicon RNA as described in Example 3, PCR amplification was performed using neomycin resistance gene-specific primers; sense primer, NEO-S3: 5'-

AACAAGATGGATTGCACGCA-3' (SEQ ID NO: 16) and antisense primer, NEO-R: 5'-CGTCAAGAAGGCGATAGAAG-3' (SEQ ID NO: 17), and the host cellular genomic DNA extracted from each of the cell clones as a template, in order to confirm that the resistance of each of the cell clones against G418 was not due to the incorporation of the neomycin resistance gene into the genome. The cell clones used herein were the cell clones Nos. 1 to 8 obtained by retransfection of rSGREP-JFH1-derived replicated replicon RNA (rSGREP-JFH1-derived cell clones Nos. 1 to 8), and cell clones Nos. 1 to 6 obtained by retransfection of rSGREP-JCH1-derived replicated replicon RNA (rSGREP-JCH1-derived cell clones Nos. 1 to 6). As a result, as shown in Fig. 9, in the eight examined rSGREP-JFH1-derived cell clones, positive clones showing the amplification of the neomycin resistance gene were not observed. For rSGREP-JCH1-derived cell clones, only 1 out of the 6 examined clones was positive (in Fig. 9, lane 3 in the right photograph). It was considered that this positive clone had acquired G418 resistance by the incorporation of the neomycin resistance gene in rSGREP-JCH1-derived replicated replicon RNA into the genomic DNA of the host cells. Thus, in the positive clone, unlike other clones, it was thought that the replicon RNA itself did not autonomously replicate within the cells. This was confirmed by the results of the experiment shown in the next (H) that no HCV proteins were detected from the positive clone.

[0090]

(H) Detection of HCV protein

Protein was extracted from rSGREP-JFH1- and rSGREP-JCH1-transfected cell clones by a standard procedure, and then analyzed by SDS-PAGE and Western blot method (Fig. 10). The examined cell clones were the same as those used in (G) above: rSGREP-JFH1-derived cell clones Nos. 1 to 8 and rSGREP-JCH1-derived cell clones Nos. 1 to 6. In addition, a cellular extract from the cell obtained by transiently transfecting expression plasmid DNA containing NS3 gene into Huh7 cells was regarded as a positive control (NS3 protein). Furthermore, a

protein extracted from the untransfected Huh7 cells was used as a negative control. A protein sample extracted from each cell clone was blotted onto a PVDF membrane (Immobilon-P, Millipore), and then detection of NS3 protein encoded by replicated replicon RNA was performed using anti-NS3-specific antibody (provided by Dr. Moradpour; Wolk B, et al, J. Virology. 2000, 74: 2293-2304). As shown in Fig. 10, in rSGREP-JFH1-derived cell clones Nos. 1 to 8 and rSGREP-JCH1-derived cell clones Nos. 1, 2 and 4 to 6, proteins of the same size as those of the positive control were detected. In rSGREP-JCH1-derived cell clone No. 3 (the clone detected as a positive clone in (G) above), no expression of NS3 protein was detected. That is, in rSGREP-JCH1-derived cell clone No. 3, no replication of replicon RNA was confirmed. NS3 protein was not detected in the untransfected Huh7 cells, revealing that in cell clones wherein NS3 protein was detected, the transfected replicon RNA autonomously replicated so that NS3 protein was expressed.

[0091]

Moreover, by the use of the serum of a hepatitis C patient as an antibody, the expression of NS5a protein from the replicon RNA was also confirmed in each cell clone for which the expression of NS3 protein had been confirmed as described above.

[0092]

Based on the results of (G) and (H) above, it was confirmed that replicon RNAs were replicated in the cell clones established by transfection of the replicon RNA.

[0093]

[Example 5]

(I) Analysis of adaptive mutation

According to descriptions of Example 3, total RNA obtained from the replicon-replicating cell clones established through the transfection of rSGREP-JFH1 into Huh7 cells was re-transfected to another Huh7 cells, thereby

establishing 21 cell clones. Total RNA was extracted from each of these cell clones by a standard procedure. cDNA corresponding to the replicon RNA was synthesized using the total RNA as a template, reverse transcriptase Superscript II (Invitrogen) and primer 9641R-IH (5'-GCACTCTCTGCAGTCATGCGGCTCACGGAC-3' (SEQ ID NO: 18)). The composition of a reaction solution for the synthesis of cDNA by reverse transcription reaction is as shown below.

[0094]

Composition of Reaction Solution	Fluid Volume (μl)
5x 1st strand Buffer	4
2 mM dNTP	5
0.1 M DTT	1
9651R-IH primer (100 μM)	1
DW (distilled water)	6.5
Sample RNA (2 mg/mL)	1
RNasin (Promega) (40 U/μL)	0.5
Superscript II RT (Invitrogen)	1
Total	20 μl

[0095]

In cDNA synthesis reaction, the above reagents other than RNasin and Superscript II were mixed to prepare a first reaction solution. The solution was heated at 90°C for 3 minutes, and then cooled on ice. Subsequently, RNasin and Superscript II were added to this reaction solution, and then the solution was allowed to react at 42°C for 1 hour, followed by another reaction at 70°C for 15 minutes.

[0096]

Furthermore, PCR amplification was performed using the thus obtained cDNA together with five primer sets by the following procedures, so that DNA amplification fragments covering almost all the regions of the replicon RNA were

obtained. The primer sets used and regions amplified by each primer set are shown in Table 1 and Table 2 below.

[0097]

[Table 1]

Designation of amplified fragment	Primer set		Amplified region
	Primer 1	Primer 2	
A/	42S-IH	433R-neo	41 - 470
B/	C/S17ssp	4680R-IH	28 - 3026
C/	4534S-IH	7279R-IH	2880 - 5625
D/	7198S-IH	9367R-IH	5544 - 7713
E/	9247S-NF	9576R-NF	7597 - 7960

[0098]

In Table 1, an amplified region is represented by nucleotide numbers in rSGREP-JFH1 (SEQ ID NO: 1) that the region corresponds to.

[0099]

[Table 2]

Primer designation	Nucleotide sequence (5'→3')	SEQ ID NO:
42S-IH	CCCCTGTGAGGAACTACTGTCTTCACGC	SEQ ID NO: 19
C/S17ssp	CCGGGAGAGCCATAGTGGTCTGCG	SEQ ID NO: 20
4534S-IH	CCACTCAAAGAAAAAGTGTGACGAGCTCGC	SEQ ID NO: 21
7198S-IH	GGCTTGGGCACGGCCTGA	SEQ ID NO: 22
9247S-NF	GCGGTGAAGACCAAGCTCAAACTCACTCCA	SEQ ID NO: 23
433R-neo	AGAACCTGCGTGCAATCCATC	SEQ ID NO: 24
4680R-IH	CCCGTCATGAGGGCGTCGGTGGC	SEQ ID NO: 25
7279R-IH	ACCAGCAACGGTGGGCGGTTGGTAATC	SEQ ID NO: 26
9367R-RI	GGCACGCGACACGCTGTG	SEQ ID NO: 27
9576R-NF	AGCTAGCCGTGACTAGGGCTAAGATGGAGC	SEQ ID NO: 28

[0100]

The composition of a reaction solution in this PCR reaction is as follows.

[0101]

Composition of Reaction Solution	Fluid Volume (μl)
Primer 1 (10 μM)	1.0
Primer 2 (10 μM)	1.0
2.5 mM dNTPs	5.0
10x LA Buffer	5.0
$MgCl_2$ (25 mM)	5.0
LA Taq (TAKARA) (5 U/μl)	0.3
DW (distilled water)	30.7
Template cDNA	2.0
Total	50 μl

[0102]

In addition, PCR reaction conditions are as follows: 95°C for 2 minutes; 35 cycles of 98°C for 10 seconds and then 68°C for 8 minutes; and 72°C for 7 minutes; after which the temperature is kept at 4°C.

[0103]

The nucleotide sequence of each PCR product obtained as described above was determined, and then the RNA sequence corresponding to the DNA sequence was compared with the sequence of rSGREP-JFH1. The results are shown in Table 3.

[0104]

[Table 3]

Region	Synonymous substitution	Nonsynonymous substitution	Total number of mutations
NS3	0	5	5
NS4A	0	2	2
NS4B	0	3	3
NS5A	0	7	7
NS5B	3	5	8
Total	3	22	25

[0105]

As shown in Table 3, total number of nucleotide mutations observed in 21 cell clones was 25. 22 of these mutations were nonsynonymous substitutions inducing amino acid mutation. Types of these mutations are as shown in Table 4. In addition, the positions of these mutations in the non-structural region are shown in Fig. 11.

[0106]

[Table 4]

Clone	Mutation site			
designation	Nucleotide No.	Nucleotide mutation	Amino acid mutation	Amino acid No.
C1	7098	A ⇒ G	None	
	7157	A ⇒ G	Y ⇒ C	2824
C2	4955	C ⇒ U	A ⇒ V	2090
C3	4936	A ⇒ G	T ⇒ A	2084
	5000	A ⇒ G	Y ⇒ C	2105
	7287	A ⇒ G	None	
	7288	A ⇒ G	M ⇒ V	2868
C4	5901	G ⇒ U	E ⇒ D	2405
	6113	A ⇒ U	H ⇒ L	2476
C5	2890	A ⇒ G	K ⇒ E	1402
C6	7209	A ⇒ G	None	

[0107]

In Table 4 and Fig. 11, "C1 to C6" represent replicon-replicating cell clones C1 to C6 having replicon RNA found to have mutations. "Nucleotide No." shows the corresponding nucleotide numbers within the nucleotide sequence of replicon RNA rSGREP-JFH1 (SEQ ID NO: 1). "Amino acid No." shows the corresponding amino acid numbers within the amino acid sequence encoded by the JFH-1 clone (SEQ ID NO: 4). The types of nucleotides and amino acids at

mutation sites are described according to their general notations. As shown in Table 4, in clone C2, a nucleotide corresponding to nucleotide No. 4955 of SEQ ID NO: 1 on the replicon RNA mutated from C (cytosine) to U (uracil), which results in a mutation of an amino acid corresponding to amino acid No. 2090 of SEQ ID NO: 4 from A (alanine) to V (valine).

[0108]

Furthermore, mutation positions shown in Fig. 11 are shown with bar lines with the nucleotide numbers shown in Table 4. A thick bar line represents nonsynonymous substitution, and a thin bar line represents synonymous substitution.

[0109]

There were 2 clones having no nucleotide mutations at all that cause amino acid mutations. When Northern blot analysis was conducted for the 2 clones, it was shown that in these 2 clones, the quantity of replicon RNAs replicated was lower than those in the cell clones that had replicated replicon RNAs having a nucleotide mutation that causes an amino acid mutation. Hence, it was considered that the nucleotide mutation causing an amino acid mutation within the replicon RNA was an adaptive mutation for increasing the replication efficiency of the replicon RNA in Huh7 cells.

[0110]

[Effects of Invention]

According to the present invention, an HCV-RNA replicon derived from the genotype 2a strain of HCV was obtained for the first time. The replicon-replicating cell according to the present invention can be used as a culture system for the continuous production of RNA and HCV proteins derived from HCV of genotype 2a. Furthermore, the replicon-replicating cell according to the present invention is useful as a test system for screening for various substances that affect HCV replication and/or the translation of HCV proteins.

[0111]

[Sequence Listing]

SEQUENCE LISTING

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Tokyo Metropolitan Organization for Medical Research
Johannes Gutenberg-Universitaet Mainz

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<223> Inventor: Wakita, Takaji
Inventor: Kato, Takanobu
Inventor: Date, Tomoko

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Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Thr Thr Arg Lys Thr Ser
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Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro Ile Pro Lys Asp Arg
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Arg Ser Thr Gly Lys Ala Trp Gly Lys Pro Gly Arg Pro Trp Pro Leu
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Tyr Gly Asn Glu Gly Leu Gly Trp Ala Gly Trp Leu Leu Ser Pro Arg
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ggc tct cgc ccc tcc tgg ggc ccc act gac ccc cgg cat agg tcg cgc   691
Gly Ser Arg Pro Ser Trp Gly Pro Thr Asp Pro Arg His Arg Ser Arg
           105                 110                 115

aac gtg ggt aaa gtc atc gac acc cta acg tgt ggc ttt gcc gac ctc   739
Asn Val Gly Lys Val Ile Asp Thr Leu Thr Cys Gly Phe Ala Asp Leu
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atg ggg tac atc ccc gtc gta ggc gcc ccg ctt agt ggc gcc gcc aga   787
Met Gly Tyr Ile Pro Val Val Gly Ala Pro Leu Ser Gly Ala Ala Arg
    135                 140                 145

gct gtc gcg cac ggc gtg aga gtc ctg gag gac ggg gtt aat tat gca   835
Ala Val Ala His Gly Val Arg Val Leu Glu Asp Gly Val Asn Tyr Ala
150                 155                 160                 165

aca ggg aac cta ccc ggt ttc ccc ttt tct atc ttc ttg ctg gcc ctg   883
Thr Gly Asn Leu Pro Gly Phe Pro Phe Ser Ile Phe Leu Leu Ala Leu
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ttg tcc tgc atc acc gtt ccg gtc tct gct gcc cag gtg aag aat acc   931
Leu Ser Cys Ile Thr Val Pro Val Ser Ala Ala Gln Val Lys Asn Thr
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Trp Gln Leu Glu Ala Ala Val Leu His Val Pro Gly Cys Val Pro Cys
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gag aga gtg ggg aat acg tca cgg tgt tgg gtg cca gtc tcg cca aac   1075
Glu Arg Val Gly Asn Thr Ser Arg Cys Trp Val Pro Val Ser Pro Asn
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atg gct gtg cgg cag ccc ggt gcc ctc acg cag ggt ctg cgg acg cac   1123
Met Ala Val Arg Gln Pro Gly Ala Leu Thr Gln Gly Leu Arg Thr His
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atc gat atg gtt gtg atg tcc gcc acc ttc tgc tct gct ctc tac gtg   1171
Ile Asp Met Val Val Met Ser Ala Thr Phe Cys Ser Ala Leu Tyr Val
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ggg gac ctc tgt ggc ggg gtg atg ctc gcg gcc cag gtg ttc atc gtc   1219
Gly Asp Leu Cys Gly Gly Val Met Leu Ala Ala Gln Val Phe Ile Val
       280                 285                 290

tcg ccg cag tac cac tgg ttt gtg caa gaa tgc aat tgc tcc atc tac   1267
Ser Pro Gln Tyr His Trp Phe Val Gln Glu Cys Asn Cys Ser Ile Tyr
    295                 300                 305

cct ggc acc atc act gga cac cgc atg gca tgg gac atg atg atg aac   1315
Pro Gly Thr Ile Thr Gly His Arg Met Ala Trp Asp Met Met Met Asn
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Trp Ser Pro Thr Ala Thr Met Ile Leu Ala Tyr Val Met Arg Val Pro
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gag gtc atc ata gac atc gtt agc ggg gct cac tgg ggc gtc atg ttc   1411
Glu Val Ile Ile Asp Ile Val Ser Gly Ala His Trp Gly Val Met Phe
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ggc ttg gcc tac ttc tct atg cag gga gcg tgg gcg aag gtc att gtc   1459
Gly Leu Ala Tyr Phe Ser Met Gln Gly Ala Trp Ala Lys Val Ile Val
        360                 365                 370

atc ctt ctg ctg gcc gct ggg gtg gac gcg ggc acc acc acc gtt gga   1507
Ile Leu Leu Leu Ala Ala Gly Val Asp Ala Gly Thr Thr Thr Val Gly
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ggc gct gtt gca cgt tcc acc aac gtg att gcc ggc gtg ttc agc cat   1555
Gly Ala Val Ala Arg Ser Thr Asn Val Ile Ala Gly Val Phe Ser His
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Gly Pro Gln Gln Asn Ile Gln Leu Ile Asn Thr Asn Gly Ser Trp His
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ctc gcg gcc ttg ttc tac acc aac cgc ttt aac tcg tca ggg tgt cca   1699
Leu Ala Ala Leu Phe Tyr Thr Asn Arg Phe Asn Ser Ser Gly Cys Pro
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ggc acc cta cag tac gag gat aat gtc acc aat cca gag gat atg agg   1795
Gly Thr Leu Gln Tyr Glu Asp Asn Val Thr Asn Pro Glu Asp Met Arg
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ccg tac tgc tgg cac tac ccc cca aag ccg tgt ggc gta gtc ccc gcg   1843
Pro Tyr Cys Trp His Tyr Pro Pro Lys Pro Cys Gly Val Val Pro Ala
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agg tct gtg tgt ggc cca gtg tac tgt ttc acc ccc agc ccg gta gta   1891
Arg Ser Val Cys Gly Pro Val Tyr Cys Phe Thr Pro Ser Pro Val Val
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gtg ggc acg acc gac aga cgt gga gtg ccc acc tac aca tgg gga gag   1939
Val Gly Thr Thr Asp Arg Arg Gly Val Pro Thr Tyr Thr Trp Gly Glu
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aat gag aca gat gtc ttc cta ctg aac agc acc cga ccg ccg cag ggc   1987
Asn Glu Thr Asp Val Phe Leu Leu Asn Ser Thr Arg Pro Pro Gln Gly
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Ser Trp Phe Gly Cys Thr Trp Met Asn Ser Thr Gly Phe Thr Lys Thr
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tgt ggc gcg cca cct tgc cgc acc aga gct gac ttc aac gcc agc acg   2083
Cys Gly Ala Pro Pro Cys Arg Thr Arg Ala Asp Phe Asn Ala Ser Thr
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Asp Leu Leu Cys Pro Thr Asp Cys Phe Arg Lys His Pro Asp Ala Thr
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tat att aag tgt ggt tct ggg ccc tgg ctc aca cca aag tgc ctg gtc   2179
Tyr Ile Lys Cys Gly Ser Gly Pro Trp Leu Thr Pro Lys Cys Leu Val
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cac tac cct tac aga ctc tgg cat tac ccc tgc aca gtc aat ttt acc   2227
His Tyr Pro Tyr Arg Leu Trp His Tyr Pro Cys Thr Val Asn Phe Thr
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atc ttc aag ata aga atg tat gta ggg ggg gtt gag cac agg ctc acg   2275
Ile Phe Lys Ile Arg Met Tyr Val Gly Gly Val Glu His Arg Leu Thr
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gcc gca tgc aac ttc act cgt ggg gat cgc tgc gac ttg gag gac agg   2323
Ala Ala Cys Asn Phe Thr Arg Gly Asp Arg Cys Asp Leu Glu Asp Arg
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gac agg agt cag ctg tct cct ctg ttg cac tct acc acg gaa tgg gcc   2371
Asp Arg Ser Gln Leu Ser Pro Leu Leu His Ser Thr Thr Glu Trp Ala
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ctc cac ctt cac cag aac atc gtg gac gta caa tac atg tat ggc ctc   2467
Leu His Leu His Gln Asn Ile Val Asp Val Gln Tyr Met Tyr Gly Leu
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```

```
tca cct gct atc aca aaa tac gtc gtt cga tgg gag tgg gtg gta ctc   2515
Ser Pro Ala Ile Thr Lys Tyr Val Val Arg Trp Glu Trp Val Val Leu
710                 715                 720                 725

tta ttc ctg ctc tta gcg gac gcc aga gtc tgc gcc tgc ttg tgg atg   2563
Leu Phe Leu Leu Leu Ala Asp Ala Arg Val Cys Ala Cys Leu Trp Met
                730                 735                 740

ctc atc ttg ttg ggc cag gcc gaa gca gca ttg gag aag ttg gtc gtc   2611
Leu Ile Leu Leu Gly Gln Ala Glu Ala Ala Leu Glu Lys Leu Val Val
            745                 750                 755

ttg cac gct gcg agt gcg gct aac tgc cat ggc ctc cta tat ttt gcc   2659
Leu His Ala Ala Ser Ala Ala Asn Cys His Gly Leu Leu Tyr Phe Ala
        760                 765                 770

atc ttc ttc gtg gca gct tgg cac atc agg ggt cgg gtg gtc ccc ttg   2707
Ile Phe Phe Val Ala Ala Trp His Ile Arg Gly Arg Val Val Pro Leu
    775                 780                 785

acc acc tat tgc ctc act ggc cta tgg ccc ttc tgc cta ctg ctc atg   2755
Thr Thr Tyr Cys Leu Thr Gly Leu Trp Pro Phe Cys Leu Leu Leu Met
790                 795                 800                 805

gca ctg ccc cgg cag gct tat gcc tat gac gca cct gtg cac gga cag   2803
Ala Leu Pro Arg Gln Ala Tyr Ala Tyr Asp Ala Pro Val His Gly Gln
                810                 815                 820

ata ggc gtg ggt ttg ttg ata ttg atc acc ctc ttc aca ctc acc ccg   2851
Ile Gly Val Gly Leu Leu Ile Leu Ile Thr Leu Phe Thr Leu Thr Pro
            825                 830                 835
```

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ggg tat aag acc ctc ctc ggc cag tgt ctg tgg tgg ttg tgc tat ctc   2899
Gly Tyr Lys Thr Leu Leu Gly Gln Cys Leu Trp Trp Leu Cys Tyr Leu
       840                 845                 850

ctg acc ctg ggg gaa gcc atg att cag gag tgg gta cca ccc atg cag   2947
Leu Thr Leu Gly Glu Ala Met Ile Gln Glu Trp Val Pro Pro Met Gln
    855                 860                 865

gtg cgc ggc ggc cgc gat ggc atc gcg tgg gcc gtc act ata ttc tgc   2995
Val Arg Gly Gly Arg Asp Gly Ile Ala Trp Ala Val Thr Ile Phe Cys
870                 875                 880                 885

ccg ggt gtg gtg ttt gac att acc aaa tgg ctt ttg gcg ttg ctt ggg   3043
Pro Gly Val Val Phe Asp Ile Thr Lys Trp Leu Leu Ala Leu Leu Gly
                890                 895                 900

cct gct tac ctc tta agg gcc gct ttg aca cat gtg ccg tac ttc gtc   3091
Pro Ala Tyr Leu Leu Arg Ala Ala Leu Thr His Val Pro Tyr Phe Val
          905                 910                 915

aga gct cac gct ctg ata agg gta tgc gct ttg gtg aag cag ctc gcg   3139
Arg Ala His Ala Leu Ile Arg Val Cys Ala Leu Val Lys Gln Leu Ala
       920                 925                 930

ggg ggt agg tat gtt cag gtg gcg cta ttg gcc ctt ggc agg tgg act   3187
Gly Gly Arg Tyr Val Gln Val Ala Leu Leu Ala Leu Gly Arg Trp Thr
    935                 940                 945

ggc acc tac atc tat gac cac ctc aca cct atg tcg gac tgg gcc gct   3235
Gly Thr Tyr Ile Tyr Asp His Leu Thr Pro Met Ser Asp Trp Ala Ala
950                 955                 960                 965
```

```
agc ggc ctg cgc gac tta gcg gtc gcc gtg gaa ccc atc atc ttc agt   3283
Ser Gly Leu Arg Asp Leu Ala Val Ala Val Glu Pro Ile Ile Phe Ser
                970                 975                 980

ccg atg gag aag aag gtc atc gtc tgg gga gcg gag acg gct gca tgt   3331
Pro Met Glu Lys Lys Val Ile Val Trp Gly Ala Glu Thr Ala Ala Cys
            985                 990                 995

ggg gac att cta cat gga ctt ccc gtg tcc gcc cga ctc ggc cag gag   3379
Gly Asp Ile Leu His Gly Leu Pro Val Ser Ala Arg Leu Gly Gln Glu
        1000                1005                1010

atc ctc ctc ggc cca gct gat ggc tac acc tcc aag ggg tgg aag ctc   3427
Ile Leu Leu Gly Pro Ala Asp Gly Tyr Thr Ser Lys Gly Trp Lys Leu
    1015                1020                1025

ctt gct ccc atc act gct tat gcc cag caa aca cga ggc ctc ctg ggc   3475
Leu Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu Gly
1030                1035                1040                1045

gcc ata gtg gtg agt atg acg ggg cgt gac agg aca gaa cag gcc ggg   3523
Ala Ile Val Val Ser Met Thr Gly Arg Asp Arg Thr Glu Gln Ala Gly
                1050                1055                1060

gaa gtc caa atc ctg tcc aca gtc tct cag tcc ttc ctc gga aca acc   3571
Glu Val Gln Ile Leu Ser Thr Val Ser Gln Ser Phe Leu Gly Thr Thr
            1065                1070                1075

atc tcg ggg gtt ttg tgg act gtt tac cac gga gct ggc aac aag act   3619
Ile Ser Gly Val Leu Trp Thr Val Tyr His Gly Ala Gly Asn Lys Thr
        1080                1085                1090
```

```
cta gcc ggc tta cgg ggt ccg gtc acg cag atg tac tcg agt gct gag   3667
Leu Ala Gly Leu Arg Gly Pro Val Thr Gln Met Tyr Ser Ser Ala Glu
  1095              1100              1105

ggg gac ttg gta ggc tgg ccc agc ccc cct ggg acc aag tct ttg gag   3715
Gly Asp Leu Val Gly Trp Pro Ser Pro Pro Gly Thr Lys Ser Leu Glu
1110              1115              1120              1125

ccg tgc aag tgt gga gcc gtc gac cta tat ctg gtc acg cgg aac gct   3763
Pro Cys Lys Cys Gly Ala Val Asp Leu Tyr Leu Val Thr Arg Asn Ala
             1130              1135              1140

gat gtc atc ccg gct cgg aga cgc ggg gac aag cgg gga gca ttg ctc   3811
Asp Val Ile Pro Ala Arg Arg Arg Gly Asp Lys Arg Gly Ala Leu Leu
         1145              1150              1155

tcc ccg aga ccc att tcg acc ttg aag ggg tcc tcg ggg ggg ccg gtg   3859
Ser Pro Arg Pro Ile Ser Thr Leu Lys Gly Ser Ser Gly Gly Pro Val
      1160              1165              1170

ctc tgc cct agg ggc cac gtc gtt ggg ctc ttc cga gca gct gtg tgc   3907
Leu Cys Pro Arg Gly His Val Val Gly Leu Phe Arg Ala Ala Val Cys
  1175              1180              1185

tct cgg ggc gtg gcc aaa tcc atc gat ttc atc ccc gtt gag aca ctc   3955
Ser Arg Gly Val Ala Lys Ser Ile Asp Phe Ile Pro Val Glu Thr Leu
1190              1195              1200              1205

gac gtt gtt aca agg tct ccc act ttc agt gac aac agc acg cca ccg   4003
Asp Val Val Thr Arg Ser Pro Thr Phe Ser Asp Asn Ser Thr Pro Pro
             1210              1215              1220
```

```
gct gtg ccc cag acc tat cag gtc ggg tac ttg cat gct cca act ggc   4051
Ala Val Pro Gln Thr Tyr Gln Val Gly Tyr Leu His Ala Pro Thr Gly
          1225              1230              1235

agt gga aag agc acc aag gtc cct gtc gcg tat gcc gcc cag ggg tac   4099
Ser Gly Lys Ser Thr Lys Val Pro Val Ala Tyr Ala Ala Gln Gly Tyr
      1240              1245              1250

aaa gta cta gtg ctt aac ccc tcg gta gct gcc acc ctg ggg ttt ggg   4147
Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala Thr Leu Gly Phe Gly
   1255              1260              1265

gcg tac cta tcc aag gca cat ggc atc aat ccc aac att agg act gga   4195
Ala Tyr Leu Ser Lys Ala His Gly Ile Asn Pro Asn Ile Arg Thr Gly
1270              1275              1280              1285

gtc agg acc gtg atg acc ggg gag gcc atc acg tac tcc aca tat ggc   4243
Val Arg Thr Val Met Thr Gly Glu Ala Ile Thr Tyr Ser Thr Tyr Gly
             1290              1295              1300

aaa ttt ctc gcc gat ggg ggc tgc gct agc ggc gcc tat gac atc atc   4291
Lys Phe Leu Ala Asp Gly Gly Cys Ala Ser Gly Ala Tyr Asp Ile Ile
          1305              1310              1315

ata tgc gat gaa tgc cac gct gtg gat gct acc tcc att ctc ggc atc   4339
Ile Cys Asp Glu Cys His Ala Val Asp Ala Thr Ser Ile Leu Gly Ile
      1320              1325              1330

gga acg gtc ctt gat caa gca gag aca gcc ggg gtc aga cta act gtg   4387
Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Val Arg Leu Thr Val
   1335              1340              1345
```

```
ctg gct acg gcc aca ccc ccc ggg tca gtg aca acc ccc cat ccc gat   4435
Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Thr Pro His Pro Asp
1350             1355             1360             1365

ata gaa gag gta ggc ctc ggg cgg gag ggt gag atc ccc ttc tat ggg   4483
Ile Glu Glu Val Gly Leu Gly Arg Glu Gly Glu Ile Pro Phe Tyr Gly
             1370             1375             1380

agg gcg att ccc cta tcc tgc atc aag gga ggg aga cac ctg att ttc   4531
Arg Ala Ile Pro Leu Ser Cys Ile Lys Gly Gly Arg His Leu Ile Phe
         1385             1390             1395

tgc cac tca aag aaa aag tgt gac gag ctc gcg gcg gcc ctt cgg ggc   4579
Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Ala Leu Arg Gly
     1400             1405             1410

atg ggc ttg aat gcc gtg gca tac tat aga ggg ttg gac gtc tcc ata   4627
Met Gly Leu Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Ile
 1415             1420             1425

ata cca gct cag gga gat gtg gtg gtc gtc gcc acc gac gcc ctc atg   4675
Ile Pro Ala Gln Gly Asp Val Val Val Val Ala Thr Asp Ala Leu Met
1430             1435             1440             1445

acg ggg tac act gga gac ttt gac tcc gtg atc gac tgc aat gta gcg   4723
Thr Gly Tyr Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Val Ala
             1450             1455             1460

gtc acc caa gct gtc gac ttc agc ctg gac ccc acc ttc act ata acc   4771
Val Thr Gln Ala Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Thr
         1465             1470             1475
```

```
aca cag act gtc cca caa gac gct gtc tca cgc agt cag cgc cgc ggg   4819
Thr Gln Thr Val Pro Gln Asp Ala Val Ser Arg Ser Gln Arg Arg Gly
      1480              1485              1490

cgc aca ggt aga gga aga cag ggc act tat agg tat gtt tcc act ggt   4867
Arg Thr Gly Arg Gly Arg Gln Gly Thr Tyr Arg Tyr Val Ser Thr Gly
  1495              1500              1505

gaa cga gcc tca gga atg ttt gac agt gta gtg ctt tgt gag tgc tac   4915
Glu Arg Ala Ser Gly Met Phe Asp Ser Val Val Leu Cys Glu Cys Tyr
1510              1515              1520              1525

gac gca ggg gct gcg tgg tac gat ctc aca cca gcg gag acc acc gtc   4963
Asp Ala Gly Ala Ala Trp Tyr Asp Leu Thr Pro Ala Glu Thr Thr Val
             1530              1535              1540

agg ctt aga gcg tat ttc aac acg ccc ggc cta ccc gtg tgt caa gac   5011
Arg Leu Arg Ala Tyr Phe Asn Thr Pro Gly Leu Pro Val Cys Gln Asp
         1545              1550              1555

cat ctt gaa ttt tgg gag gca gtt ttc acc ggc ctc aca cac ata gac   5059
His Leu Glu Phe Trp Glu Ala Val Phe Thr Gly Leu Thr His Ile Asp
      1560              1565              1570

gcc cac ttc ctc tcc caa aca aag caa gcg ggg gag aac ttc gcg tac   5107
Ala His Phe Leu Ser Gln Thr Lys Gln Ala Gly Glu Asn Phe Ala Tyr
  1575              1580              1585

cta gta gcc tac caa gct acg gtg tgc gcc aga gcc aag gcc cct ccc   5155
Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Lys Ala Pro Pro
1590              1595              1600              1605
```

```
ccg tcc tgg gac gcc atg tgg aag tgc ctg gcc cga ctc aag cct acg   5203
Pro Ser Trp Asp Ala Met Trp Lys Cys Leu Ala Arg Leu Lys Pro Thr
             1610              1615              1620

ctt gcg ggc ccc aca cct ctc ctg tac cgt ttg ggc cct att acc aat   5251
Leu Ala Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Pro Ile Thr Asn
         1625              1630              1635

gag gtc acc ctc aca cac cct ggg acg aag tac atc gcc aca tgc atg   5299
Glu Val Thr Leu Thr His Pro Gly Thr Lys Tyr Ile Ala Thr Cys Met
      1640              1645              1650

caa gct gac ctt gag gtc atg acc agc acg tgg gtc cta gct gga gga   5347
Gln Ala Asp Leu Glu Val Met Thr Ser Thr Trp Val Leu Ala Gly Gly
   1655              1660              1665

gtc ctg gca gcc gtc gcc gca tat tgc ctg gcg act gga tgc gtt tcc   5395
Val Leu Ala Ala Val Ala Ala Tyr Cys Leu Ala Thr Gly Cys Val Ser
1670              1675              1680              1685

atc atc ggc cgc ttg cac gtc aac cag cga gtc gtc gtt gcg ccg gat   5443
Ile Ile Gly Arg Leu His Val Asn Gln Arg Val Val Val Ala Pro Asp
             1690              1695              1700

aag gag gtc ctg tat gag gct ttt gat gag atg gag gaa tgc gcc tct   5491
Lys Glu Val Leu Tyr Glu Ala Phe Asp Glu Met Glu Glu Cys Ala Ser
         1705              1710              1715

agg gcg gct ctc atc gaa gag ggg cag cgg ata gcc gag atg ttg aag   5539
Arg Ala Ala Leu Ile Glu Glu Gly Gln Arg Ile Ala Glu Met Leu Lys
      1720              1725              1730
```

```
tcc aag atc caa ggc ttg ctg cag cag gcc tct aag cag gcc cag gac   5587
Ser Lys Ile Gln Gly Leu Leu Gln Gln Ala Ser Lys Gln Ala Gln Asp
   1735              1740              1745

ata caa ccc gct atg cag gct tca tgg ccc aaa gtg gaa caa ttt tgg   5635
Ile Gln Pro Ala Met Gln Ala Ser Trp Pro Lys Val Glu Gln Phe Trp
1750              1755              1760              1765

gcc aga cac atg tgg aac ttc att agc ggc atc caa tac ctc gca gga   5683
Ala Arg His Met Trp Asn Phe Ile Ser Gly Ile Gln Tyr Leu Ala Gly
             1770              1775              1780

ttg tca aca ctg cca ggg aac ccc gcg gtg gct tcc atg atg gca ttc   5731
Leu Ser Thr Leu Pro Gly Asn Pro Ala Val Ala Ser Met Met Ala Phe
          1785              1790              1795

agt gcc gcc ctc acc agt ccg ttg tcg acc agt acc acc atc ctt ctc   5779
Ser Ala Ala Leu Thr Ser Pro Leu Ser Thr Ser Thr Thr Ile Leu Leu
      1800              1805              1810

aac atc atg gga ggc tgg tta gcg tcc cag atc gca cca ccc gcg ggg   5827
Asn Ile Met Gly Gly Trp Leu Ala Ser Gln Ile Ala Pro Pro Ala Gly
   1815              1820              1825

gcc acc ggc ttt gtc gtc agt ggc ctg gtg ggg gct gcc gtg ggc agc   5875
Ala Thr Gly Phe Val Val Ser Gly Leu Val Gly Ala Ala Val Gly Ser
1830              1835              1840              1845

ata ggc ctg ggt aag gtg ctg gtg gac atc ctg gca gga tat ggt gcg   5923
Ile Gly Leu Gly Lys Val Leu Val Asp Ile Leu Ala Gly Tyr Gly Ala
             1850              1855              1860
```

```
ggc att tcg ggg gcc ctc gtc gca ttc aag atc atg tct ggc gag aag   5971
Gly Ile Ser Gly Ala Leu Val Ala Phe Lys Ile Met Ser Gly Glu Lys
          1865              1870              1875

ccc tct atg gaa gat gtc atc aat cta ctg cct ggg atc ctg tct ccg   6019
Pro Ser Met Glu Asp Val Ile Asn Leu Leu Pro Gly Ile Leu Ser Pro
      1880              1885              1890

gga gcc ctg gtg gtg ggg gtc atc tgc gcg gcc att ctg cgc cgc cac   6067
Gly Ala Leu Val Val Gly Val Ile Cys Ala Ala Ile Leu Arg Arg His
   1895              1900              1905

gtg gga ccg ggg gag ggc gcg gtc caa tgg atg aac agg ctt att gcc   6115
Val Gly Pro Gly Glu Gly Ala Val Gln Trp Met Asn Arg Leu Ile Ala
1910              1915              1920              1925

ttt gct tcc aga gga aac cac gtc gcc cct act cac tac gtg acg gag   6163
Phe Ala Ser Arg Gly Asn His Val Ala Pro Thr His Tyr Val Thr Glu
             1930              1935              1940

tcg gat gcg tcg cag cgt gtg acc caa cta ctt ggc tct ctt act ata   6211
Ser Asp Ala Ser Gln Arg Val Thr Gln Leu Leu Gly Ser Leu Thr Ile
          1945              1950              1955

acc agc cta ctc aga aga ctc cac aat tgg ata act gag gac tgc ccc   6259
Thr Ser Leu Leu Arg Arg Leu His Asn Trp Ile Thr Glu Asp Cys Pro
      1960              1965              1970

atc cca tgc tcc gga tcc tgg ctc cgc gac gtg tgg gac tgg gtt tgc   6307
Ile Pro Cys Ser Gly Ser Trp Leu Arg Asp Val Trp Asp Trp Val Cys
   1975              1980              1985
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```
acc atc ttg aca gac ttc aaa aat tgg ctg acc tct aaa ttg ttc ccc   6355
Thr Ile Leu Thr Asp Phe Lys Asn Trp Leu Thr Ser Lys Leu Phe Pro
1990             1995             2000             2005

aag ctg ccc ggc ctc ccc ttc atc tct tgt caa aag ggg tac aag ggt   6403
Lys Leu Pro Gly Leu Pro Phe Ile Ser Cys Gln Lys Gly Tyr Lys Gly
             2010             2015             2020

gtg tgg gcc ggc act ggc atc atg acc acg cgc tgc cct tgc ggc gcc   6451
Val Trp Ala Gly Thr Gly Ile Met Thr Thr Arg Cys Pro Cys Gly Ala
         2025             2030             2035

aac atc tct ggc aat gtc cgc ctg ggc tct atg agg atc aca ggg cct   6499
Asn Ile Ser Gly Asn Val Arg Leu Gly Ser Met Arg Ile Thr Gly Pro
     2040             2045             2050

aaa acc tgc atg aac acc tgg cag ggg acc ttt cct atc aat tgc tac   6547
Lys Thr Cys Met Asn Thr Trp Gln Gly Thr Phe Pro Ile Asn Cys Tyr
 2055             2060             2065

acg gag ggc cag tgc gcg ccg aaa ccc ccc acg aac tac aag acc gcc   6595
Thr Glu Gly Gln Cys Ala Pro Lys Pro Pro Thr Asn Tyr Lys Thr Ala
2070             2075             2080             2085

atc tgg agg gtg gcg gcc tcg gag tac gcg gag gtg acg cag cat ggg   6643
Ile Trp Arg Val Ala Ala Ser Glu Tyr Ala Glu Val Thr Gln His Gly
             2090             2095             2100

tcg tac tcc tat gta aca gga ctg acc act gac aat ctg aaa att cct   6691
Ser Tyr Ser Tyr Val Thr Gly Leu Thr Thr Asp Asn Leu Lys Ile Pro
         2105             2110             2115
```

```
tgc caa cta cct tct cca gag ttt ttc tcc tgg gtg gac ggt gtg cag   6739
Cys Gln Leu Pro Ser Pro Glu Phe Phe Ser Trp Val Asp Gly Val Gln
      2120              2125              2130

atc cat agg ttt gca ccc aca cca aag ccg ttt ttc cgg gat gag gtc   6787
Ile His Arg Phe Ala Pro Thr Pro Lys Pro Phe Phe Arg Asp Glu Val
   2135              2140              2145

tcg ttc tgc gtt ggg ctt aat tcc tat gct gtc ggg tcc cag ctt ccc   6835
Ser Phe Cys Val Gly Leu Asn Ser Tyr Ala Val Gly Ser Gln Leu Pro
2150              2155              2160              2165

tgt gaa cct gag ccc gac gca gac gta ttg agg tcc atg cta aca gat   6883
Cys Glu Pro Glu Pro Asp Ala Asp Val Leu Arg Ser Met Leu Thr Asp
      .       2170              2175              2180

ccg ccc cac atc acg gcg gag act gcg gcg cgg cgc ttg gca cgg gga   6931
Pro Pro His Ile Thr Ala Glu Thr Ala Ala Arg Arg Leu Ala Arg Gly
         2185              2190              2195

tca cct cca tct gag gcg agc tcc tca gtg agc cag cta tca gca ccg   6979
Ser Pro Pro Ser Glu Ala Ser Ser Ser Val Ser Gln Leu Ser Ala Pro
      2200              2205              2210

tcg ctg cgg gcc acc tgc acc acc cac agc aac acc tat gac gtg gac   7027
Ser Leu Arg Ala Thr Cys Thr Thr His Ser Asn Thr Tyr Asp Val Asp
   2215              2220              2225

atg gtc gat gcc aac ctg ctc atg gag ggc ggt gtg gct cag aca gag   7075
Met Val Asp Ala Asn Leu Leu Met Glu Gly Gly Val Ala Gln Thr Glu
2230              2235              2240              2245
```

```
cct gag tcc agg gtg ccc gtt ctg gac ttt ctc gag cca atg gcc gag   7123
Pro Glu Ser Arg Val Pro Val Leu Asp Phe Leu Glu Pro Met Ala Glu
             2250              2255              2260

gaa gag agc gac ctt gag ccc tca ata cca tcg gag tgc atg ctc ccc   7171
Glu Glu Ser Asp Leu Glu Pro Ser Ile Pro Ser Glu Cys Met Leu Pro
         2265              2270              2275

agg agc ggg ttt cca cgg gcc tta ccg gct tgg gca cgg cct gac tac   7219
Arg Ser Gly Phe Pro Arg Ala Leu Pro Ala Trp Ala Arg Pro Asp Tyr
     2280              2285              2290

aac ccg ccg ctc gtg gaa tcg tgg agg agg cca gat tac caa ccg ccc   7267
Asn Pro Pro Leu Val Glu Ser Trp Arg Arg Pro Asp Tyr Gln Pro Pro
 2295              2300              2305

acc gtt gct ggt tgt gct ctc ccc ccc ccc aag aag gcc ccg acg cct   7315
Thr Val Ala Gly Cys Ala Leu Pro Pro Pro Lys Lys Ala Pro Thr Pro
2310              2315              2320              2325

ccc cca agg aga cgc cgg aca gtg ggt ctg agc gag agc acc ata tca   7363
Pro Pro Arg Arg Arg Arg Thr Val Gly Leu Ser Glu Ser Thr Ile Ser
             2330              2335              2340

gaa gcc ctc cag caa ctg gcc atc aag acc ttt ggc cag ccc ccc tcg   7411
Glu Ala Leu Gln Gln Leu Ala Ile Lys Thr Phe Gly Gln Pro Pro Ser
         2345              2350              2355

agc ggt gat gca ggc tcg tcc acg ggg gcg ggc gcc gcc gaa tcc ggc   7459
Ser Gly Asp Ala Gly Ser Ser Thr Gly Ala Gly Ala Ala Glu Ser Gly
     2360              2365              2370
```

```
ggt ccg acg tcc cct ggt gag ccg gcc ccc tca gag aca ggt tcc gcc   7507
Gly Pro Thr Ser Pro Gly Glu Pro Ala Pro Ser Glu Thr Gly Ser Ala
   2375              2380              2385

tcc tct atg ccc ccc ctc gag ggg gag cct gga gat ccg gac ctg gag   7555
Ser Ser Met Pro Pro Leu Glu Gly Glu Pro Gly Asp Pro Asp Leu Glu
2390              2395              2400              2405

tct gat cag gta gag ctt caa cct ccc ccc cag ggg ggg ggg gta gct   7603
Ser Asp Gln Val Glu Leu Gln Pro Pro Pro Gln Gly Gly Gly Val Ala
             2410              2415              2420

ccc ggt tcg ggc tcg ggg tct tgg tct act tgc tcc gag gag gac gat   7651
Pro Gly Ser Gly Ser Gly Ser Trp Ser Thr Cys Ser Glu Glu Asp Asp
          2425              2430              2435

acc acc gtg tgc tgc tcc atg tca tac tcc tgg acc ggg gct cta ata   7699
Thr Thr Val Cys Cys Ser Met Ser Tyr Ser Trp Thr Gly Ala Leu Ile
      2440              2445              2450

act ccc tgt agc ccc gaa gag gaa aag ttg cca atc aac cct ttg agt   7747
Thr Pro Cys Ser Pro Glu Glu Glu Lys Leu Pro Ile Asn Pro Leu Ser
   2455              2460              2465

aac tcg ctg ttg cga tac cat aac aag gtg tac tgt aca aca tca aag   7795
Asn Ser Leu Leu Arg Tyr His Asn Lys Val Tyr Cys Thr Thr Ser Lys
2470              2475              2480              2485

agc gcc tca cag agg gct aaa aag gta act ttt gac agg acg caa gtg   7843
Ser Ala Ser Gln Arg Ala Lys Lys Val Thr Phe Asp Arg Thr Gln Val
             2490              2495              2500
```

```
ctc gac gcc cat tat gac tca gtc tta aag gac atc aag cta gcg gct   7891
Leu Asp Ala His Tyr Asp Ser Val Leu Lys Asp Ile Lys Leu Ala Ala
          2505              2510              2515

tcc aag gtc agc gca agg ctc ctc acc ttg gag gag gcg tgc cag ttg   7939
Ser Lys Val Ser Ala Arg Leu Leu Thr Leu Glu Glu Ala Cys Gln Leu
      2520              2525              2530

act cca ccc cat tct gca aga tcc aag tat gga ttc ggg gcc aag gag   7987
Thr Pro Pro His Ser Ala Arg Ser Lys Tyr Gly Phe Gly Ala Lys Glu
   2535              2540              2545

gtc cgc agc ttg tcc ggg agg gcc gtt aac cac atc aag tcc gtg tgg   8035
Val Arg Ser Leu Ser Gly Arg Ala Val Asn His Ile Lys Ser Val Trp
2550              2555              2560              2565

aag gac ctc ctg gaa gac cca caa aca cca att ccc aca acc atc atg   8083
Lys Asp Leu Leu Glu Asp Pro Gln Thr Pro Ile Pro Thr Thr Ile Met
             2570              2575              2580

gcc aaa aat gag gtg ttc tgc gtg gac ccc gcc aag ggg ggt aag aaa   8131
Ala Lys Asn Glu Val Phe Cys Val Asp Pro Ala Lys Gly Gly Lys Lys
          2585              2590              2595

cca gct cgc ctc atc gtt tac cct gac ctc ggc gtc cgg gtc tgc gag   8179
Pro Ala Arg Leu Ile Val Tyr Pro Asp Leu Gly Val Arg Val Cys Glu
      2600              2605              2610

aaa atg gcc ctc tat gac att aca caa aag ctt cct cag gcg gta atg   8227
Lys Met Ala Leu Tyr Asp Ile Thr Gln Lys Leu Pro Gln Ala Val Met
   2615              2620              2625
```

```
gga gct tcc tat ggc ttc cag tac tcc cct gcc caa cgg gtg gag tat   8275
Gly Ala Ser Tyr Gly Phe Gln Tyr Ser Pro Ala Gln Arg Val Glu Tyr
2630             2635             2640             2645

ctc ttg aaa gca tgg gcg gaa aag aag gac ccc atg ggt ttt tcg tat   8323
Leu Leu Lys Ala Trp Ala Glu Lys Lys Asp Pro Met Gly Phe Ser Tyr
             2650             2655             2660

gat acc cga tgc ttc gac tca acc gtc act gag aga gac atc agg acc   8371
Asp Thr Arg Cys Phe Asp Ser Thr Val Thr Glu Arg Asp Ile Arg Thr
         2665             2670             2675

gag gag tcc ata tac cag gcc tgc tcc ctg ccc gag gag gcc cgc act   8419
Glu Glu Ser Ile Tyr Gln Ala Cys Ser Leu Pro Glu Glu Ala Arg Thr
     2680             2685             2690

gcc ata cac tcg ctg act gag aga ctt tac gta gga ggg ccc atg ttc   8467
Ala Ile His Ser Leu Thr Glu Arg Leu Tyr Val Gly Gly Pro Met Phe
 2695             2700             2705

aac agc aag ggt caa acc tgc ggt tac aga cgt tgc cgc gcc agc ggg   8515
Asn Ser Lys Gly Gln Thr Cys Gly Tyr Arg Arg Cys Arg Ala Ser Gly
2710             2715             2720             2725

gtg cta acc act agc atg ggt aac acc atc aca tgc tat gtg aaa gcc   8563
Val Leu Thr Thr Ser Met Gly Asn Thr Ile Thr Cys Tyr Val Lys Ala
             2730             2735             2740

cta gcg gcc tgc aag gct gcg ggg ata gtt gcg ccc aca atg ctg gta   8611
Leu Ala Ala Cys Lys Ala Ala Gly Ile Val Ala Pro Thr Met Leu Val
         2745             2750             2755
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tgc ggc gat gac cta gta gtc atc tca gaa agc cag ggg act gag gag   8659
Cys Gly Asp Asp Leu Val Val Ile Ser Glu Ser Gln Gly Thr Glu Glu
      2760              2765              2770

gac gag cgg aac ctg aga gcc ttc acg gag gcc atg acc agg tac tct   8707
Asp Glu Arg Asn Leu Arg Ala Phe Thr Glu Ala Met Thr Arg Tyr Ser
   2775              2780              2785

gcc cct cct ggt gat ccc ccc aga ccg gaa tat gac ctg gag cta ata   8755
Ala Pro Pro Gly Asp Pro Pro Arg Pro Glu Tyr Asp Leu Glu Leu Ile
2790              2795              2800              2805

aca tcc tgt tcc tca aat gtg tct gtg gcg ttg ggc ccg cgg ggc cgc   8803
Thr Ser Cys Ser Ser Asn Val Ser Val Ala Leu Gly Pro Arg Gly Arg
             2810              2815              2820

cgc aga tac tac ctg acc aga gac cca acc act cca ctc gcc cgg gct   8851
Arg Arg Tyr Tyr Leu Thr Arg Asp Pro Thr Thr Pro Leu Ala Arg Ala
          2825              2830              2835

gcc tgg gaa aca gtt aga cac tcc cct atc aat tca tgg ctg gga aac   8899
Ala Trp Glu Thr Val Arg His Ser Pro Ile Asn Ser Trp Leu Gly Asn
      2840              2845              2850

atc atc cag tat gct cca acc ata tgg gtt cgc atg gtc cta atg aca   8947
Ile Ile Gln Tyr Ala Pro Thr Ile Trp Val Arg Met Val Leu Met Thr
   2855              2860              2865

cac ttc ttc tcc att ctc atg gtc caa gac acc ctg gac cag aac ctc   8995
His Phe Phe Ser Ile Leu Met Val Gln Asp Thr Leu Asp Gln Asn Leu
2870              2875              2880              2885
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aac ttt gag atg tat gga tca gta tac tcc gtg aat cct ttg gac ctt   9043
Asn Phe Glu Met Tyr Gly Ser Val Tyr Ser Val Asn Pro Leu Asp Leu
             2890              2895              2900

cca gcc ata att gag agg tta cac ggg ctt gac gcc ttt tct atg cac   9091
Pro Ala Ile Ile Glu Arg Leu His Gly Leu Asp Ala Phe Ser Met His
         2905              2910              2915

aca tac tct cac cac gaa ctg acg cgg gtg gct tca gcc ctc aga aaa   9139
Thr Tyr Ser His His Glu Leu Thr Arg Val Ala Ser Ala Leu Arg Lys
      2920              2925              2930

ctt ggg gcg cca ccc ctc agg gtg tgg aag agt cgg gct cgc gca gtc   9187
Leu Gly Ala Pro Pro Leu Arg Val Trp Lys Ser Arg Ala Arg Ala Val
   2935              2940              2945

agg gcg tcc ctc atc tcc cgt gga ggg aaa gcg gcc gtt tgc ggc cga   9235
Arg Ala Ser Leu Ile Ser Arg Gly Gly Lys Ala Ala Val Cys Gly Arg
2950              2955              2960              2965

tat ctc ttc aat tgg gcg gtg aag acc aag ctc aaa ctc act cca ttg   9283
Tyr Leu Phe Asn Trp Ala Val Lys Thr Lys Leu Lys Leu Thr Pro Leu
             2970              2975              2980

ccg gag gcg cgc cta ctg gac tta tcc agt tgg ttc acc gtc ggc gcc   9331
Pro Glu Ala Arg Leu Leu Asp Leu Ser Ser Trp Phe Thr Val Gly Ala
         2985              2990              2995

ggc ggg ggc gac att ttt cac agc gtg tcg cgc gcc cga ccc cgc tca   9379
Gly Gly Gly Asp Ile Phe His Ser Val Ser Arg Ala Arg Pro Arg Ser
      3000              3005              3010
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tta ctc ttc ggc cta ctc cta ctt ttc gta ggg gta ggc ctc ttc cta 9427
Leu Leu Phe Gly Leu Leu Leu Leu Phe Val Gly Val Gly Leu Phe Leu
3015 3020 3025

ctc ccc gct cgg tag agcggcacac actaggtaca ctccatagct aactgttcct 9482
Leu Pro Ala Arg
3030

ttttttttt ttttttttt ttttttttt ttttttttt ttcttttttt ttttttcccc 9542

tctttcttcc cttctcatct tattctactt tctttcttgg tggctccatc ttagccctag 9602

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Gly Val Tyr Leu Leu Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Thr
35 40 45
Thr Arg Lys Thr Ser Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro
50 55 60

```
Ile Pro Lys Asp Arg Arg Ser Thr Gly Lys Ala Trp Gly Lys Pro Gly
 65                  70                  75                  80
Arg Pro Trp Pro Leu Tyr Gly Asn Glu Gly Leu Gly Trp Ala Gly Trp
                85                  90                  95
Leu Leu Ser Pro Arg Gly Ser Arg Pro Ser Trp Gly Pro Thr Asp Pro
           100                 105                 110
Arg His Arg Ser Arg Asn Val Gly Lys Val Ile Asp Thr Leu Thr Cys
       115                 120                 125
Gly Phe Ala Asp Leu Met Gly Tyr Ile Pro Val Val Gly Ala Pro Leu
    130                 135                 140
Ser Gly Ala Ala Arg Ala Val Ala His Gly Val Arg Val Leu Glu Asp
145                 150                 155                 160
Gly Val Asn Tyr Ala Thr Gly Asn Leu Pro Gly Phe Pro Phe Ser Ile
               165                 170                 175
Phe Leu Leu Ala Leu Leu Ser Cys Ile Thr Val Pro Val Ser Ala Ala
           180                 185                 190
Gln Val Lys Asn Thr Ser Ser Ser Tyr Met Val Thr Asn Asp Cys Ser
       195                 200                 205
Asn Asp Ser Ile Thr Trp Gln Leu Glu Ala Ala Val Leu His Val Pro
    210                 215                 220
Gly Cys Val Pro Cys Glu Arg Val Gly Asn Thr Ser Arg Cys Trp Val
225                 230                 235                 240
Pro Val Ser Pro Asn Met Ala Val Arg Gln Pro Gly Ala Leu Thr Gln
               245                 250                 255
Gly Leu Arg Thr His Ile Asp Met Val Val Met Ser Ala Thr Phe Cys
           260                 265                 270
Ser Ala Leu Tyr Val Gly Asp Leu Cys Gly Gly Val Met Leu Ala Ala
       275                 280                 285
Gln Val Phe Ile Val Ser Pro Gln Tyr His Trp Phe Val Gln Glu Cys
    290                 295                 300
Asn Cys Ser Ile Tyr Pro Gly Thr Ile Thr Gly His Arg Met Ala Trp
305                 310                 315                 320
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Asp Met Met Met Asn Trp Ser Pro Thr Ala Thr Met Ile Leu Ala Tyr
                325                 330                 335
Val Met Arg Val Pro Glu Val Ile Ile Asp Ile Val Ser Gly Ala His
            340                 345                 350
Trp Gly Val Met Phe Gly Leu Ala Tyr Phe Ser Met Gln Gly Ala Trp
        355                 360                 365
Ala Lys Val Ile Val Ile Leu Leu Leu Ala Ala Gly Val Asp Ala Gly
    370                 375                 380
Thr Thr Thr Val Gly Gly Ala Val Ala Arg Ser Thr Asn Val Ile Ala
385                 390                 395                 400
Gly Val Phe Ser His Gly Pro Gln Gln Asn Ile Gln Leu Ile Asn Thr
                405                 410                 415
Asn Gly Ser Trp His Ile Asn Arg Thr Ala Leu Asn Cys Asn Asp Ser
            420                 425                 430
Leu Asn Thr Gly Phe Leu Ala Ala Leu Phe Tyr Thr Asn Arg Phe Asn
        435                 440                 445
Ser Ser Gly Cys Pro Gly Arg Leu Ser Ala Cys Arg Asn Ile Glu Ala
    450                 455                 460
Phe Arg Ile Gly Trp Gly Thr Leu Gln Tyr Glu Asp Asn Val Thr Asn
465                 470                 475                 480
Pro Glu Asp Met Arg Pro Tyr Cys Trp His Tyr Pro Pro Lys Pro Cys
                485                 490                 495
Gly Val Val Pro Ala Arg Ser Val Cys Gly Pro Val Tyr Cys Phe Thr
            500                 505                 510
Pro Ser Pro Val Val Val Gly Thr Thr Asp Arg Arg Gly Val Pro Thr
        515                 520                 525
Tyr Thr Trp Gly Glu Asn Glu Thr Asp Val Phe Leu Leu Asn Ser Thr
    530                 535                 540
Arg Pro Pro Gln Gly Ser Trp Phe Gly Cys Thr Trp Met Asn Ser Thr
545                 550                 555                 560
Gly Phe Thr Lys Thr Cys Gly Ala Pro Pro Cys Arg Thr Arg Ala Asp
                565                 570                 575
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Phe Asn Ala Ser Thr Asp Leu Leu Cys Pro Thr Asp Cys Phe Arg Lys
580 585 590
His Pro Asp Ala Thr Tyr Ile Lys Cys Gly Ser Gly Pro Trp Leu Thr
595 600 605
Pro Lys Cys Leu Val His Tyr Pro Tyr Arg Leu Trp His Tyr Pro Cys
610 615 620
Thr Val Asn Phe Thr Ile Phe Lys Ile Arg Met Tyr Val Gly Gly Val
625 630 635 640
Glu His Arg Leu Thr Ala Ala Cys Asn Phe Thr Arg Gly Asp Arg Cys
645 650 655
Asp Leu Glu Asp Arg Asp Arg Ser Gln Leu Ser Pro Leu Leu His Ser
660 665 670
Thr Thr Glu Trp Ala Ile Leu Pro Cys Thr Tyr Ser Asp Leu Pro Ala
675 680 685
Leu Ser Thr Gly Leu Leu His Leu His Gln Asn Ile Val Asp Val Gln
690 695 700
Tyr Met Tyr Gly Leu Ser Pro Ala Ile Thr Lys Tyr Val Val Arg Trp
705 710 715 720
Glu Trp Val Val Leu Leu Phe Leu Leu Leu Ala Asp Ala Arg Val Cys
725 730 735
Ala Cys Leu Trp Met Leu Ile Leu Leu Gly Gln Ala Glu Ala Ala Leu
740 745 750
Glu Lys Leu Val Val Leu His Ala Ala Ser Ala Ala Asn Cys His Gly
755 760 765
Leu Leu Tyr Phe Ala Ile Phe Phe Val Ala Ala Trp His Ile Arg Gly
770 775 780
Arg Val Val Pro Leu Thr Thr Tyr Cys Leu Thr Gly Leu Trp Pro Phe
785 790 795 800
Cys Leu Leu Leu Met Ala Leu Pro Arg Gln Ala Tyr Ala Tyr Asp Ala
805 810 815
Pro Val His Gly Gln Ile Gly Val Gly Leu Leu Ile Leu Ile Thr Leu
820 825 830

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Phe Thr Leu Thr Pro Gly Tyr Lys Thr Leu Leu Gly Gln Cys Leu Trp
       835                 840                 845
Trp Leu Cys Tyr Leu Leu Thr Leu Gly Glu Ala Met Ile Gln Glu Trp
    850                 855                 860
Val Pro Pro Met Gln Val Arg Gly Gly Arg Asp Gly Ile Ala Trp Ala
865                 870                 875                 880
Val Thr Ile Phe Cys Pro Gly Val Val Phe Asp Ile Thr Lys Trp Leu
                885                 890                 895
Leu Ala Leu Leu Gly Pro Ala Tyr Leu Leu Arg Ala Ala Leu Thr His
           900                 905                 910
Val Pro Tyr Phe Val Arg Ala His Ala Leu Ile Arg Val Cys Ala Leu
       915                 920                 925
Val Lys Gln Leu Ala Gly Gly Arg Tyr Val Gln Val Ala Leu Leu Ala
    930                 935                 940
Leu Gly Arg Trp Thr Gly Thr Tyr Ile Tyr Asp His Leu Thr Pro Met
945                 950                 955                 960
Ser Asp Trp Ala Ala Ser Gly Leu Arg Asp Leu Ala Val Ala Val Glu
                965                 970                 975
Pro Ile Ile Phe Ser Pro Met Glu Lys Lys Val Ile Val Trp Gly Ala
           980                 985                 990
Glu Thr Ala Ala Cys Gly Asp Ile Leu His Gly Leu Pro Val Ser Ala
       995                1000                1005
Arg Leu Gly Gln Glu Ile Leu Leu Gly Pro Ala Asp Gly Tyr Thr Ser
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Lys Gly Trp Lys Leu Leu Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr
1025                1030                1035                1040
Arg Gly Leu Leu Gly Ala Ile Val Val Ser Met Thr Gly Arg Asp Arg
                1045                1050                1055
Thr Glu Gln Ala Gly Glu Val Gln Ile Leu Ser Thr Val Ser Gln Ser
           1060                1065                1070
Phe Leu Gly Thr Thr Ile Ser Gly Val Leu Trp Thr Val Tyr His Gly
       1075                1080                1085
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Ala Gly Asn Lys Thr Leu Ala Gly Leu Arg Gly Pro Val Thr Gln Met
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Tyr Ser Ser Ala Glu Gly Asp Leu Val Gly Trp Pro Ser Pro Pro Gly
1105 1110 1115 1120
Thr Lys Ser Leu Glu Pro Cys Lys Cys Gly Ala Val Asp Leu Tyr Leu
1125 1130 1135
Val Thr Arg Asn Ala Asp Val Ile Pro Ala Arg Arg Arg Gly Asp Lys
1140 1145 1150
Arg Gly Ala Leu Leu Ser Pro Arg Pro Ile Ser Thr Leu Lys Gly Ser
1155 1160 1165
Ser Gly Gly Pro Val Leu Cys Pro Arg Gly His Val Val Gly Leu Phe
1170 1175 1180
Arg Ala Ala Val Cys Ser Arg Gly Val Ala Lys Ser Ile Asp Phe Ile
1185 1190 1195 1200
Pro Val Glu Thr Leu Asp Val Val Thr Arg Ser Pro Thr Phe Ser Asp
1205 1210 1215
Asn Ser Thr Pro Pro Ala Val Pro Gln Thr Tyr Gln Val Gly Tyr Leu
1220 1225 1230
His Ala Pro Thr Gly Ser Gly Lys Ser Thr Lys Val Pro Val Ala Tyr
1235 1240 1245
Ala Ala Gln Gly Tyr Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala
1250 1255 1260
Thr Leu Gly Phe Gly Ala Tyr Leu Ser Lys Ala His Gly Ile Asn Pro
1265 1270 1275 1280
Asn Ile Arg Thr Gly Val Arg Thr Val Met Thr Gly Glu Ala Ile Thr
1285 1290 1295
Tyr Ser Thr Tyr Gly Lys Phe Leu Ala Asp Gly Gly Cys Ala Ser Gly
1300 1305 1310
Ala Tyr Asp Ile Ile Ile Cys Asp Glu Cys His Ala Val Asp Ala Thr
1315 1320 1325
Ser Ile Leu Gly Ile Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly
1330 1335 1340

Val Arg Leu Thr Val Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr
1345 1350 1355 1360
Thr Pro His Pro Asp Ile Glu Glu Val Gly Leu Gly Arg Glu Gly Glu
1365 1370 1375
Ile Pro Phe Tyr Gly Arg Ala Ile Pro Leu Ser Cys Ile Lys Gly Gly
1380 1385 1390
Arg His Leu Ile Phe Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala
1395 1400 1405
Ala Ala Leu Arg Gly Met Gly Leu Asn Ala Val Ala Tyr Tyr Arg Gly
1410 1415 1420
Leu Asp Val Ser Ile Ile Pro Ala Gln Gly Asp Val Val Val Val Ala
1425 1430 1435 1440
Thr Asp Ala Leu Met Thr Gly Tyr Thr Gly Asp Phe Asp Ser Val Ile
1445 1450 1455
Asp Cys Asn Val Ala Val Thr Gln Ala Val Asp Phe Ser Leu Asp Pro
1460 1465 1470
Thr Phe Thr Ile Thr Thr Gln Thr Val Pro Gln Asp Ala Val Ser Arg
1475 1480 1485
Ser Gln Arg Arg Gly Arg Thr Gly Arg Gly Arg Gln Gly Thr Tyr Arg
1490 1495 1500
Tyr Val Ser Thr Gly Glu Arg Ala Ser Gly Met Phe Asp Ser Val Val
1505 1510 1515 1520
Leu Cys Glu Cys Tyr Asp Ala Gly Ala Ala Trp Tyr Asp Leu Thr Pro
1525 1530 1535
Ala Glu Thr Thr Val Arg Leu Arg Ala Tyr Phe Asn Thr Pro Gly Leu
1540 1545 1550
Pro Val Cys Gln Asp His Leu Glu Phe Trp Glu Ala Val Phe Thr Gly
1555 1560 1565
Leu Thr His Ile Asp Ala His Phe Leu Ser Gln Thr Lys Gln Ala Gly
1570 1575 1580
Glu Asn Phe Ala Tyr Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg
1585 1590 1595 1600

Ala Lys Ala Pro Pro Pro Ser Trp Asp Ala Met Trp Lys Cys Leu Ala
1605 1610 1615

Arg Leu Lys Pro Thr Leu Ala Gly Pro Thr Pro Leu Leu Tyr Arg Leu
1620 1625 1630

Gly Pro Ile Thr Asn Glu Val Thr Leu Thr His Pro Gly Thr Lys Tyr
1635 1640 1645

Ile Ala Thr Cys Met Gln Ala Asp Leu Glu Val Met Thr Ser Thr Trp
1650 1655 1660

Val Leu Ala Gly Gly Val Leu Ala Ala Val Ala Ala Tyr Cys Leu Ala
1665 1670 1675 1680

Thr Gly Cys Val Ser Ile Ile Gly Arg Leu His Val Asn Gln Arg Val
1685 1690 1695

Val Val Ala Pro Asp Lys Glu Val Leu Tyr Glu Ala Phe Asp Glu Met
1700 1705 1710

Glu Glu Cys Ala Ser Arg Ala Ala Leu Ile Glu Glu Gly Gln Arg Ile
1715 1720 1725

Ala Glu Met Leu Lys Ser Lys Ile Gln Gly Leu Leu Gln Gln Ala Ser
1730 1735 1740

Lys Gln Ala Gln Asp Ile Gln Pro Ala Met Gln Ala Ser Trp Pro Lys
1745 1750 1755 1760

Val Glu Gln Phe Trp Ala Arg His Met Trp Asn Phe Ile Ser Gly Ile
1765 1770 1775

Gln Tyr Leu Ala Gly Leu Ser Thr Leu Pro Gly Asn Pro Ala Val Ala
1780 1785 1790

Ser Met Met Ala Phe Ser Ala Ala Leu Thr Ser Pro Leu Ser Thr Ser
1795 1800 1805

Thr Thr Ile Leu Leu Asn Ile Met Gly Gly Trp Leu Ala Ser Gln Ile
1810 1815 1820

Ala Pro Pro Ala Gly Ala Thr Gly Phe Val Val Ser Gly Leu Val Gly
1825 1830 1835 1840

Ala Ala Val Gly Ser Ile Gly Leu Gly Lys Val Leu Val Asp Ile Leu
1845 1850 1855

Ala Gly Tyr Gly Ala Gly Ile Ser Gly Ala Leu Val Ala Phe Lys Ile
1860 1865 1870

Met Ser Gly Glu Lys Pro Ser Met Glu Asp Val Ile Asn Leu Leu Pro
1875 1880 1885

Gly Ile Leu Ser Pro Gly Ala Leu Val Val Gly Val Ile Cys Ala Ala
1890 1895 1900

Ile Leu Arg Arg His Val Gly Pro Gly Glu Gly Ala Val Gln Trp Met
1905 1910 1915 1920

Asn Arg Leu Ile Ala Phe Ala Ser Arg Gly Asn His Val Ala Pro Thr
1925 1930 1935

His Tyr Val Thr Glu Ser Asp Ala Ser Gln Arg Val Thr Gln Leu Leu
1940 1945 1950

Gly Ser Leu Thr Ile Thr Ser Leu Leu Arg Arg Leu His Asn Trp Ile
1955 1960 1965

Thr Glu Asp Cys Pro Ile Pro Cys Ser Gly Ser Trp Leu Arg Asp Val
1970 1975 1980

Trp Asp Trp Val Cys Thr Ile Leu Thr Asp Phe Lys Asn Trp Leu Thr
1985 1990 1995 2000

Ser Lys Leu Phe Pro Lys Leu Pro Gly Leu Pro Phe Ile Ser Cys Gln
2005 2010 2015

Lys Gly Tyr Lys Gly Val Trp Ala Gly Thr Gly Ile Met Thr Thr Arg
2020 2025 2030

Cys Pro Cys Gly Ala Asn Ile Ser Gly Asn Val Arg Leu Gly Ser Met
2035 2040 2045

Arg Ile Thr Gly Pro Lys Thr Cys Met Asn Thr Trp Gln Gly Thr Phe
2050 2055 2060

Pro Ile Asn Cys Tyr Thr Glu Gly Gln Cys Ala Pro Lys Pro Pro Thr
2065 2070 2075 2080

Asn Tyr Lys Thr Ala Ile Trp Arg Val Ala Ala Ser Glu Tyr Ala Glu
2085 2090 2095

Val Thr Gln His Gly Ser Tyr Ser Tyr Val Thr Gly Leu Thr Thr Asp
2100 2105 2110

Asn Leu Lys Ile Pro Cys Gln Leu Pro Ser Pro Glu Phe Phe Ser Trp
2115 2120 2125

Val Asp Gly Val Gln Ile His Arg Phe Ala Pro Thr Pro Lys Pro Phe
2130 2135 2140

Phe Arg Asp Glu Val Ser Phe Cys Val Gly Leu Asn Ser Tyr Ala Val
2145 2150 2155 2160

Gly Ser Gln Leu Pro Cys Glu Pro Glu Pro Asp Ala Asp Val Leu Arg
2165 2170 2175

Ser Met Leu Thr Asp Pro Pro His Ile Thr Ala Glu Thr Ala Ala Arg
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Arg Leu Ala Arg Gly Ser Pro Pro Ser Glu Ala Ser Ser Ser Val Ser
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Gln Leu Ser Ala Pro Ser Leu Arg Ala Thr Cys Thr Thr His Ser Asn
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Thr Tyr Asp Val Asp Met Val Asp Ala Asn Leu Leu Met Glu Gly Gly
2225 2230 2235 2240

Val Ala Gln Thr Glu Pro Glu Ser Arg Val Pro Val Leu Asp Phe Leu
2245 2250 2255

Glu Pro Met Ala Glu Glu Glu Ser Asp Leu Glu Pro Ser Ile Pro Ser
2260 2265 2270

Glu Cys Met Leu Pro Arg Ser Gly Phe Pro Arg Ala Leu Pro Ala Trp
2275 2280 2285

Ala Arg Pro Asp Tyr Asn Pro Pro Leu Val Glu Ser Trp Arg Arg Pro
2290 2295 2300

Asp Tyr Gln Pro Pro Thr Val Ala Gly Cys Ala Leu Pro Pro Pro Lys
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Lys Ala Pro Thr Pro Pro Pro Arg Arg Arg Arg Thr Val Gly Leu Ser
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Glu Ser Thr Ile Ser Glu Ala Leu Gln Gln Leu Ala Ile Lys Thr Phe
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Gly Gln Pro Pro Ser Ser Gly Asp Ala Gly Ser Ser Thr Gly Ala Gly
2355 2360 2365

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Ser Glu Glu Asp Asp Thr Thr Val Cys Cys Ser Met Ser Tyr Ser Trp
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Cys Thr Thr Ser Lys Ser Ala Ser Gln Arg Ala Lys Lys Val Thr Phe
2485 2490 2495
Asp Arg Thr Gln Val Leu Asp Ala His Tyr Asp Ser Val Leu Lys Asp
2500 2505 2510
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Lys Gly Gly Lys Lys Pro Ala Arg Leu Ile Val Tyr Pro Asp Leu Gly
2595 2600 2605
Val Arg Val Cys Glu Lys Met Ala Leu Tyr Asp Ile Thr Gln Lys Leu
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Pro Gln Ala Val Met Gly Ala Ser Tyr Gly Phe Gln Tyr Ser Pro Ala
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Gln Arg Val Glu Tyr Leu Leu Lys Ala Trp Ala Glu Lys Lys Asp Pro
2645 2650 2655
Met Gly Phe Ser Tyr Asp Thr Arg Cys Phe Asp Ser Thr Val Thr Glu
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Arg Asp Ile Arg Thr Glu Glu Ser Ile Tyr Gln Ala Cys Ser Leu Pro
2675 2680 2685
Glu Glu Ala Arg Thr Ala Ile His Ser Leu Thr Glu Arg Leu Tyr Val
2690 2695 2700
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Cys Arg Ala Ser Gly Val Leu Thr Thr Ser Met Gly Asn Thr Ile Thr
2725 2730 2735
Cys Tyr Val Lys Ala Leu Ala Ala Cys Lys Ala Ala Gly Ile Val Ala
2740 2745 2750
Pro Thr Met Leu Val Cys Gly Asp Asp Leu Val Val Ile Ser Glu Ser
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Gln Gly Thr Glu Glu Asp Glu Arg Asn Leu Arg Ala Phe Thr Glu Ala
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Met Thr Arg Tyr Ser Ala Pro Pro Gly Asp Pro Pro Arg Pro Glu Tyr
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Asp Leu Glu Leu Ile Thr Ser Cys Ser Ser Asn Val Ser Val Ala Leu
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Gly Pro Arg Gly Arg Arg Arg Tyr Tyr Leu Thr Arg Asp Pro Thr Thr
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2850 2855 2860
Met Val Leu Met Thr His Phe Phe Ser Ile Leu Met Val Gln Asp Thr
2865 2870 2875 2880

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Asn Pro Leu Asp Leu Pro Ala Ile Ile Glu Arg Leu His Gly Leu Asp
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Ala Phe Ser Met His Thr Tyr Ser His His Glu Leu Thr Arg Val Ala
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Ser Ala Leu Arg Lys Leu Gly Ala Pro Pro Leu Arg Val Trp Lys Ser
   2930                2935                2940
Arg Ala Arg Ala Val Arg Ala Ser Leu Ile Ser Arg Gly Gly Lys Ala
2945                2950                2955                2960
Ala Val Cys Gly Arg Tyr Leu Phe Asn Trp Ala Val Lys Thr Lys Leu
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Lys Leu Thr Pro Leu Pro Glu Ala Arg Leu Leu Asp Leu Ser Ser Trp
         2980                2985                2990
Phe Thr Val Gly Ala Gly Gly Gly Asp Ile Phe His Ser Val Ser Arg
      2995                3000                3005
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aagactgggt cctttcttgg ataaacccac tctatgcccg gccatttggg cgtgcccccg 240

caagactgct agccgagtag cgttgggttg cgaaaggcct tgtggtactg cctgataggg 300

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Met Ser Thr Asn Pro
1 5

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10 15 20

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Val Lys Phe Pro Gly Gly Gly Gln Ile Val Gly Gly Val Tyr Leu Leu
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Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Ala Thr Arg Lys Ala Ser
40 45 50

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Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro Ile Pro Lys His Arg
55 60 65

cgc tcc act ggc aag tcc tgg ggg aag cca gga tac ccc tgg ccc ctg 595
Arg Ser Thr Gly Lys Ser Trp Gly Lys Pro Gly Tyr Pro Trp Pro Leu

70 75 80 85

tat ggg aat gag ggg ctc ggt tgg gca gga tgg ctc ctg tcc cct cga 643
Tyr Gly Asn Glu Gly Leu Gly Trp Ala Gly Trp Leu Leu Ser Pro Arg
90 95 100

ggt tcc cgt ccc tca tgg ggc ccc aat gac ccc cgg cat agg tcg cgc 691
Gly Ser Arg Pro Ser Trp Gly Pro Asn Asp Pro Arg His Arg Ser Arg
105 110 115

aat gtg ggt aag gtc atc gat acc cta acg tgc ggc ttt gcc gac ctc 739
Asn Val Gly Lys Val Ile Asp Thr Leu Thr Cys Gly Phe Ala Asp Leu
120 125 130

ttg ggg tac gtc ccc gtc gta ggc gcc ccg ctt agt ggc gtt gcc agt 787
Leu Gly Tyr Val Pro Val Val Gly Ala Pro Leu Ser Gly Val Ala Ser
135 140 145

gct ctc gcg cac ggc gtg aga gtc ctg gag gac ggg gtt aat ttt gca 835
Ala Leu Ala His Gly Val Arg Val Leu Glu Asp Gly Val Asn Phe Ala
150 155 160 165

aca ggg aac tta cct ggt tgc tcc ttt tct atc ttc ttg ctg gcc cta 883
Thr Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile Phe Leu Leu Ala Leu
170 175 180

ctg tcc tgc atc act act ccg gtc tct gct gtc caa gtg aag aac acc 931
Leu Ser Cys Ile Thr Thr Pro Val Ser Ala Val Gln Val Lys Asn Thr
185 190 195

agc aac gcc tat atg gcg act aac gac tgt tcc aat gac agc atc act 979
Ser Asn Ala Tyr Met Ala Thr Asn Asp Cys Ser Asn Asp Ser Ile Thr

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       200                 205                 210

tgg cag ctt gag gcc gca gtc ctc cat gtc ccc ggg tgc gtc ccg tgc   1027
Trp Gln Leu Glu Ala Ala Val Leu His Val Pro Gly Cys Val Pro Cys
    215                 220                 225

gag aaa atg ggg aac aca tca cgg tgc tgg ata cca gtc tca cca aac   1075
Glu Lys Met Gly Asn Thr Ser Arg Cys Trp Ile Pro Val Ser Pro Asn
230                 235                 240                 245

gtg gct gtg cgg cag cct ggc gcc ctc acg cgg ggc ttg cgg acg cac   1123
Val Ala Val Arg Gln Pro Gly Ala Leu Thr Arg Gly Leu Arg Thr His
                250                 255                 260

atc gac atg gtc gtg ttg tcc gcc acg ctc tgc tcc gct ctc tac gtg   1171
Ile Asp Met Val Val Leu Ser Ala Thr Leu Cys Ser Ala Leu Tyr Val
          265                 270                 275

ggg gac ctc tgt ggc ggg gtg atg ctc gcg tcc cag atg ttc att gtc   1219
Gly Asp Leu Cys Gly Gly Val Met Leu Ala Ser Gln Met Phe Ile Val
       280                 285                 290

tcg ccg cag cac cac tgg ttc gtg cag gaa tgc aat tgc tcc atc tac   1267
Ser Pro Gln His His Trp Phe Val Gln Glu Cys Asn Cys Ser Ile Tyr
    295                 300                 305

cct ggc gcc atc act ggg cac cgt atg gca tgg gac atg atg atg aac   1315
Pro Gly Ala Ile Thr Gly His Arg Met Ala Trp Asp Met Met Met Asn
310                 315                 320                 325

tgg tcg ccc acg acc acc atg atc ctg gcg tac gtg atg cgc gtt ccc   1363
Trp Ser Pro Thr Thr Thr Met Ile Leu Ala Tyr Val Met Arg Val Pro
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                330                 335                 340

gag gtc atc ata gac atc att agc gga gct cac tgg ggc gtc atg ttt   1411
Glu Val Ile Ile Asp Ile Ile Ser Gly Ala His Trp Gly Val Met Phe
          345                 350                 355

ggc ctg gcc tac ttc tct atg cag gga gcg tgg gcg aag gtc gtt gtc   1459
Gly Leu Ala Tyr Phe Ser Met Gln Gly Ala Trp Ala Lys Val Val Val
       360                 365                 370

atc ctc ctg ctg gcc tct ggg gtg gac gcg tac acc acc acg act ggg   1507
Ile Leu Leu Leu Ala Ser Gly Val Asp Ala Tyr Thr Thr Thr Thr Gly
   375                 380                 385

agc gct gct ggg cgc act acc agt agc ctg gcc agc gcc ttc tcc cct   1555
Ser Ala Ala Gly Arg Thr Thr Ser Ser Leu Ala Ser Ala Phe Ser Pro
390                 395                 400                 405

ggc gct cgg cag aac att cag ctc att aat acc aat ggt agc tgg cac   1603
Gly Ala Arg Gln Asn Ile Gln Leu Ile Asn Thr Asn Gly Ser Trp His
              410                 415                 420

atc aac cgc acc gcc ctg aat tgc aac gat tcc ttg cac acc ggc ttc   1651
Ile Asn Arg Thr Ala Leu Asn Cys Asn Asp Ser Leu His Thr Gly Phe
          425                 430                 435

ttc acg gcc ctg ttc tac atc cat aag ttc aac tcg tcg gga tgt ccc   1699
Phe Thr Ala Leu Phe Tyr Ile His Lys Phe Asn Ser Ser Gly Cys Pro
       440                 445                 450

gag cgc ctg tcc gcc tgt cgc aac atc gag gac ttc cgg ata gga tgg   1747
Glu Arg Leu Ser Ala Cys Arg Asn Ile Glu Asp Phe Arg Ile Gly Trp
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    455                 460                 465

ggc gcc ctg caa tac gac gac aat gtc acc aat cca gaa gat atg agg   1795
Gly Ala Leu Gln Tyr Asp Asp Asn Val Thr Asn Pro Glu Asp Met Arg
470                 475                 480                 485

cca tat tgc tgg cac tac cca cca aaa cag tgt ggc gta gtc ccc gca   1843
Pro Tyr Cys Trp His Tyr Pro Pro Lys Gln Cys Gly Val Val Pro Ala
                490                 495                 500

ggg acc gtg tgc ggc cca gtg tac tgt ttc acc cct agc ccg gtg gta   1891
Gly Thr Val Cys Gly Pro Val Tyr Cys Phe Thr Pro Ser Pro Val Val
            505                 510                 515

gtg ggc acg acc gat aga ctt gga gtg cct act tac acg tgg gga gag   1939
Val Gly Thr Thr Asp Arg Leu Gly Val Pro Thr Tyr Thr Trp Gly Glu
        520                 525                 530

aat gag aca gat gtc ttc cta ttg aac agc acc cga cca ccg tcg ggg   1987
Asn Glu Thr Asp Val Phe Leu Leu Asn Ser Thr Arg Pro Pro Ser Gly
    535                 540                 545

tca tgg ttt ggc tgc acg tgg atg aac tcc act ggc ttc acc aag acc   2035
Ser Trp Phe Gly Cys Thr Trp Met Asn Ser Thr Gly Phe Thr Lys Thr
550                 555                 560                 565

tgc ggc gca cca ccc tgc cgc act aga gct gac ttc aat acc agc aca   2083
Cys Gly Ala Pro Pro Cys Arg Thr Arg Ala Asp Phe Asn Thr Ser Thr
                570                 575                 580

gat ctg ttg tgc ccc acg gac tgt ttt aga aaa cat cct gaa gcc act   2131
Asp Leu Leu Cys Pro Thr Asp Cys Phe Arg Lys His Pro Glu Ala Thr
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          585                 590                 595

tac atc aaa tgt ggt tcc ggg cct tgg ctc acg cca aag tgt ctg gtt   2179
Tyr Ile Lys Cys Gly Ser Gly Pro Trp Leu Thr Pro Lys Cys Leu Val
       600                 605                 610

gac tac ccc tac agg ctc tgg cat tac cct tgc aca gtc aat tac tcc   2227
Asp Tyr Pro Tyr Arg Leu Trp His Tyr Pro Cys Thr Val Asn Tyr Ser
    615                 620                 625

acc ttc aag atc agg atg tat gtg ggg gga gtt gag cac agg ctc atg   2275
Thr Phe Lys Ile Arg Met Tyr Val Gly Gly Val Glu His Arg Leu Met
630                 635                 640                 645

gcc gcg tgc aat ttc act cgt ggg gat cgc tgc aac ttg gag gat agg   2323
Ala Ala Cys Asn Phe Thr Arg Gly Asp Arg Cys Asn Leu Glu Asp Arg
                650                 655                 660

gac aga agt caa cag act cct ctg ttg cac tcc acc acg gaa tgg gcc   2371
Asp Arg Ser Gln Gln Thr Pro Leu Leu His Ser Thr Thr Glu Trp Ala
          665                 670                 675

att ttg ccc tgc tct ttc tca gac ttg ccc gct ttg tcg act ggt ctt   2419
Ile Leu Pro Cys Ser Phe Ser Asp Leu Pro Ala Leu Ser Thr Gly Leu
       680                 685                 690

ctc cac ctc cac caa aat atc gtg gac gta caa tat atg tat ggc ctg   2467
Leu His Leu His Gln Asn Ile Val Asp Val Gln Tyr Met Tyr Gly Leu
    695                 700                 705

tca cct gcc ctc aca caa tat atc gtt cga tgg gag tgg gta gta ctc   2515
Ser Pro Ala Leu Thr Gln Tyr Ile Val Arg Trp Glu Trp Val Val Leu
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710                 715                 720                 725

tta ttc ctg ctc cta gcg gac gcc agg gtc tgc gcc tgc ttg tgg atg   2563
Leu Phe Leu Leu Leu Ala Asp Ala Arg Val Cys Ala Cys Leu Trp Met
                730                 735                 740

ctc atc ttg ctg ggc caa gcc gaa gca gca ctg gag aag ctg gtc gtc   2611
Leu Ile Leu Leu Gly Gln Ala Glu Ala Ala Leu Glu Lys Leu Val Val
            745                 750                 755

ttg cac gct gcg agc gca gct agc tgc aat ggc ttc ctg tat ttt gtc   2659
Leu His Ala Ala Ser Ala Ala Ser Cys Asn Gly Phe Leu Tyr Phe Val
        760                 765                 770

atc ttt ctc gtg gct gct tgg cac atc aag ggt agg gtg gtc ccc ttg   2707
Ile Phe Leu Val Ala Ala Trp His Ile Lys Gly Arg Val Val Pro Leu
    775                 780                 785

gct gct tat tcc ctt act ggc ctg tgg ccg ttc tgc cta ctg ctc cta   2755
Ala Ala Tyr Ser Leu Thr Gly Leu Trp Pro Phe Cys Leu Leu Leu Leu
790                 795                 800                 805

gca ctg ccc cag cag gct tac gcc tat gat gca tct gtg cac gga cag   2803
Ala Leu Pro Gln Gln Ala Tyr Ala Tyr Asp Ala Ser Val His Gly Gln
                810                 815                 820

gtg ggc gcg gct ttg cta gta ctg att acc ctc ttt aca ctc acc ccg   2851
Val Gly Ala Ala Leu Leu Val Leu Ile Thr Leu Phe Thr Leu Thr Pro
            825                 830                 835

ggg tat aag acc ctt ctc agc cag tcc ctg tgg tgg ttg tgc tat ctc   2899
Gly Tyr Lys Thr Leu Leu Ser Gln Ser Leu Trp Trp Leu Cys Tyr Leu
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       840                 845                 850

ctg acc ctg gcg gaa acc atg gtc cag gag tgg gca cca tcc atg cag   2947
Leu Thr Leu Ala Glu Thr Met Val Gln Glu Trp Ala Pro Ser Met Gln
    855                 860                 865

gcg cgc ggc ggc cgt gat ggc atc ata tgg gcc gcc acc ata ttt tgc   2995
Ala Arg Gly Gly Arg Asp Gly Ile Ile Trp Ala Ala Thr Ile Phe Cys
870                 875                 880                 885

ccg ggc gta gtg ttt gac ata acc aag tgg ctc tta gcg gtg ctt ggg   3043
Pro Gly Val Val Phe Asp Ile Thr Lys Trp Leu Leu Ala Val Leu Gly
                890                 895                 900

cct ggt tac ctc cta aga ggt gct ttg acg cgc gtg cca tat ttc gtc   3091
Pro Gly Tyr Leu Leu Arg Gly Ala Leu Thr Arg Val Pro Tyr Phe Val
          905                 910                 915

aga gcc cac gct ctg ctg aga atg tgc act atg gtg agg cac ctc gcg   3139
Arg Ala His Ala Leu Leu Arg Met Cys Thr Met Val Arg His Leu Ala
       920                 925                 930

ggg ggt agg tac gtc cag atg gcg cta tta gcc ctt ggc agg tgg act   3187
Gly Gly Arg Tyr Val Gln Met Ala Leu Leu Ala Leu Gly Arg Trp Thr
    935                 940                 945

ggc act tac atc tat gac cac ctc acc cct atg tcg gat tgg gct gct   3235
Gly Thr Tyr Ile Tyr Asp His Leu Thr Pro Met Ser Asp Trp Ala Ala
950                 955                 960                 965

agc ggc ctg cgg gac ttg gcg gtc gct gtg gag cct atc atc ttc agt   3283
Ser Gly Leu Arg Asp Leu Ala Val Ala Val Glu Pro Ile Ile Phe Ser
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                970                 975                 980

ccg atg gag aag aaa gtc atc gtt tgg gga gcg gag acg gct gcg tgc   3331
Pro Met Glu Lys Lys Val Ile Val Trp Gly Ala Glu Thr Ala Ala Cys
          985                 990                 995

ggg gac atc ttg cac gga ctt ccc gtg tcc gcc cga ctc ggt cgg gag   3379
Gly Asp Ile Leu His Gly Leu Pro Val Ser Ala Arg Leu Gly Arg Glu
      1000                1005                1010

atc ctc ctt ggc cca gct gat ggc tac acc tcc aag ggg tgg aag ctt   3427
Ile Leu Leu Gly Pro Ala Asp Gly Tyr Thr Ser Lys Gly Trp Lys Leu
   1015                1020                1025

ctc gcc ccc atc acc gct tac gcc cag cag aca cga ggt ctc ttg ggc   3475
Leu Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu Gly
1030                1035                1040                1045

tct ata gtg gtg agc atg acg ggg cgt gac aag aca gaa cag gcc ggg   3523
Ser Ile Val Val Ser Met Thr Gly Arg Asp Lys Thr Glu Gln Ala Gly
             1050                1055                1060

gag gtc caa gtc ctg tcc aca gtc act cag tcc ttc ctc gga aca tcc   3571
Glu Val Gln Val Leu Ser Thr Val Thr Gln Ser Phe Leu Gly Thr Ser
          1065                1070                1075

att tcg ggg gtc tta tgg act gtt tac cac gga gct ggc aac aag aca   3619
Ile Ser Gly Val Leu Trp Thr Val Tyr His Gly Ala Gly Asn Lys Thr
      1080                1085                1090

cta gcc ggc tcg cgg ggc ccg gtc acg cag atg tac tcg agc gcc gag   3667
Leu Ala Gly Ser Arg Gly Pro Val Thr Gln Met Tyr Ser Ser Ala Glu
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   1095              1100              1105

ggg gac ttg gtc ggg tgg ccc agc cct cct ggg acc aaa tct ttg gag   3715
Gly Asp Leu Val Gly Trp Pro Ser Pro Pro Gly Thr Lys Ser Leu Glu
1110              1115              1120              1125

ccg tgt acg tgt gga gcg gtc gac ctg tat ttg gtc acg cgg aac gct   3763
Pro Cys Thr Cys Gly Ala Val Asp Leu Tyr Leu Val Thr Arg Asn Ala
             1130              1135              1140

gat gtc atc ccg gct cga aga cgc ggg gac aag cgg gga gcg ctg ctc   3811
Asp Val Ile Pro Ala Arg Arg Arg Gly Asp Lys Arg Gly Ala Leu Leu
          1145              1150              1155

tcc ccg aga ccc ctt tcg acc ttg aag ggg tcc tcg ggg gga cct gtg   3859
Ser Pro Arg Pro Leu Ser Thr Leu Lys Gly Ser Ser Gly Gly Pro Val
      1160              1165              1170

ctt tgc cct agg ggc cac gct gtc gga atc ttc cgg gca gct gtg tgc   3907
Leu Cys Pro Arg Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys
   1175              1180              1185

tct cgg ggt gtg gct aag tcc ata gat ttc atc ccc gtt gag acg ctc   3955
Ser Arg Gly Val Ala Lys Ser Ile Asp Phe Ile Pro Val Glu Thr Leu
1190              1195              1200              1205

gac atc gtc acg cgg tct ccc acc ttt agt gac aac agc aca cca cca   4003
Asp Ile Val Thr Arg Ser Pro Thr Phe Ser Asp Asn Ser Thr Pro Pro
             1210              1215              1220

gct gtg ccc cag acc tat cag gtg ggg tac ttg cac gcc ccc act ggc   4051
Ala Val Pro Gln Thr Tyr Gln Val Gly Tyr Leu His Ala Pro Thr Gly
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          1225              1230              1235

agt gga aaa agc acc aag gtc ccc gtc gcg tac gcc gcc cag ggg tat   4099
Ser Gly Lys Ser Thr Lys Val Pro Val Ala Tyr Ala Ala Gln Gly Tyr
       1240              1245              1250

aaa gtg ctg gtg ctc aat ccc tcg gtg gct gcc acc ctg gga ttt ggg   4147
Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala Thr Leu Gly Phe Gly
   1255              1260              1265

gcg tac ttg tcc aag gca cat ggc atc aac ccc aac att agg act gga   4195
Ala Tyr Leu Ser Lys Ala His Gly Ile Asn Pro Asn Ile Arg Thr Gly
1270              1275              1280              1285

gtc aga act gtg acg acc ggg gag ccc att aca tac tcc acg tat ggt   4243
Val Arg Thr Val Thr Thr Gly Glu Pro Ile Thr Tyr Ser Thr Tyr Gly
             1290              1295              1300

aaa ttc ctc gcc gat ggg ggc tgc gca ggc ggc gcc tat gac atc atc   4291
Lys Phe Leu Ala Asp Gly Gly Cys Ala Gly Gly Ala Tyr Asp Ile Ile
          1305              1310              1315

ata tgc gat gaa tgc cac tct gtg gat gct acc act att ctc ggc atc   4339
Ile Cys Asp Glu Cys His Ser Val Asp Ala Thr Thr Ile Leu Gly Ile
       1320              1325              1330

ggg aca gtc ctt gac caa gca gag aca gcc ggg gtc agg cta act gta   4387
Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Val Arg Leu Thr Val
   1335              1340              1345

ctg gcc acg gcc acg ccc ccc ggg tcg gtg aca acc ccc cat ccc aat   4435
Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Thr Pro His Pro Asn
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1350                1355                1360                1365

ata gag gag gta gcc ctc gga cag gag ggt gag atc ccc ttc tat ggg   4483
Ile Glu Glu Val Ala Leu Gly Gln Glu Gly Glu Ile Pro Phe Tyr Gly
                1370                1375                1380

agg gcg ttt ccc ctg tct tac atc aag gga ggg agg cac ttg att ttc   4531
Arg Ala Phe Pro Leu Ser Tyr Ile Lys Gly Gly Arg His Leu Ile Phe
            1385                1390                1395

tgc cac tca aag aaa aag tgt gac gag ctc gca acg gcc ctt cgg ggc   4579
Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Thr Ala Leu Arg Gly
        1400                1405                1410

atg ggc ttg aac gct gtg gca tat tac aga ggg ttg gac gtc tcc ata   4627
Met Gly Leu Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Ile
    1415                1420                1425

ata cca act caa gga gat gtg gtg gtc gtt gcc acc gac gcc ctc atg   4675
Ile Pro Thr Gln Gly Asp Val Val Val Val Ala Thr Asp Ala Leu Met
1430                1435                1440                1445

acg ggg tat act gga gac ttt gac tcc gtg atc gac tgc aac gta gcg   4723
Thr Gly Tyr Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Val Ala
                1450                1455                1460

gtc acc cag gcc gta gac ttc agc ctg gac ccc acc ttc act ata acc   4771
Val Thr Gln Ala Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Thr
            1465                1470                1475

aca cag act gtc ccg caa gac gct gtc tca cgt agt cag cgc cga ggg   4819
Thr Gln Thr Val Pro Gln Asp Ala Val Ser Arg Ser Gln Arg Arg Gly
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      1480              1485              1490

cgc acg ggt aga gga aga ctg ggc att tat agg tat gtt tcc act ggt   4867
Arg Thr Gly Arg Gly Arg Leu Gly Ile Tyr Arg Tyr Val Ser Thr Gly
   1495              1500              1505

gag cga gcc tca gga atg ttt gac agt gta gta ctc tgt gag tgc tac   4915
Glu Arg Ala Ser Gly Met Phe Asp Ser Val Val Leu Cys Glu Cys Tyr
1510              1515              1520              1525

gac gca gga gct gct tgg tat gag ctc tca cca gtg gag acg acc gtc   4963
Asp Ala Gly Ala Ala Trp Tyr Glu Leu Ser Pro Val Glu Thr Thr Val
             1530              1535              1540

agg ctc agg gcg tat ttc aac acg cct ggc ttg cct gtg tgc cag gac   5011
Arg Leu Arg Ala Tyr Phe Asn Thr Pro Gly Leu Pro Val Cys Gln Asp
          1545              1550              1555

cac ctt gag ttt tgg gag gca gtt ttc acc ggc ctc aca cac ata gac   5059
His Leu Glu Phe Trp Glu Ala Val Phe Thr Gly Leu Thr His Ile Asp
      1560              1565              1570

gct cat ttc ctt tcc cag aca aag cag tcg ggg gaa aat ttc gca tac   5107
Ala His Phe Leu Ser Gln Thr Lys Gln Ser Gly Glu Asn Phe Ala Tyr
   1575              1580              1585

tta gta gcc tat cag gcc aca gtg tgc gcc agg gcc aaa gcg ccc ccc   5155
Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Lys Ala Pro Pro
1590              1595              1600              1605

ccg tcc tgg gac gtc atg tgg aag tgc ttg act cga ctc aag ccc acg   5203
Pro Ser Trp Asp Val Met Trp Lys Cys Leu Thr Arg Leu Lys Pro Thr
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            1610              1615              1620

ctt gtg ggc cct aca cct ctc ctg tac cgt ttg ggc tct gtt acc aac   5251
Leu Val Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ser Val Thr Asn
          1625              1630              1635

gag gtc acc ctt aca cac ccc gtg aca aaa tac atc gcc aca tgc atg   5299
Glu Val Thr Leu Thr His Pro Val Thr Lys Tyr Ile Ala Thr Cys Met
      1640              1645              1650

caa gct gac ctc gag gtc atg acc agc acg tgg gtc ctg gct ggg gga   5347
Gln Ala Asp Leu Glu Val Met Thr Ser Thr Trp Val Leu Ala Gly Gly
   1655              1660              1665

gtc tta gca gcc gtc gcc gcg tat tgc tta gcg acc ggg tgt gtt tcc   5395
Val Leu Ala Ala Val Ala Ala Tyr Cys Leu Ala Thr Gly Cys Val Ser
1670              1675              1680              1685

atc att ggc cgt tta cac atc aac cag cga gct gtc gtc gct ccg gac   5443
Ile Ile Gly Arg Leu His Ile Asn Gln Arg Ala Val Val Ala Pro Asp
             1690              1695              1700

aag gag gtc ctc tat gag gct ttt gat gag atg gag gaa tgt gcc tcc   5491
Lys Glu Val Leu Tyr Glu Ala Phe Asp Glu Met Glu Glu Cys Ala Ser
          1705              1710              1715

aga gcg gct ctc ctt gaa gag ggg cag cgg ata gcc gag atg ctg aag   5539
Arg Ala Ala Leu Leu Glu Glu Gly Gln Arg Ile Ala Glu Met Leu Lys
      1720              1725              1730

tcc aag atc caa ggc tta ttg cag caa gcc tct aaa cag gcc cag gac   5587
Ser Lys Ile Gln Gly Leu Leu Gln Gln Ala Ser Lys Gln Ala Gln Asp
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   1735              1740              1745

ata caa ccc gct gtg caa gct tcg tgg ccc aag atg gag caa ttc tgg   5635
Ile Gln Pro Ala Val Gln Ala Ser Trp Pro Lys Met Glu Gln Phe Trp
1750              1755              1760              1765

gcc aaa cat atg tgg aac ttc ata agc ggc att cag tac ctc gca gga   5683
Ala Lys His Met Trp Asn Phe Ile Ser Gly Ile Gln Tyr Leu Ala Gly
             1770              1775              1780

ctg tca aca ctg cca ggg aac cct gct gtg gct tcc atg atg gca ttc   5731
Leu Ser Thr Leu Pro Gly Asn Pro Ala Val Ala Ser Met Met Ala Phe
          1785              1790              1795

agc gcc gcc ctc acc agt ccg ttg tca act agc acc acc atc ctt ctt   5779
Ser Ala Ala Leu Thr Ser Pro Leu Ser Thr Ser Thr Thr Ile Leu Leu
      1800              1805              1810

aac att ctg ggg ggc tgg ctg gcg tcc caa att gcg cca ccc gcg ggg   5827
Asn Ile Leu Gly Gly Trp Leu Ala Ser Gln Ile Ala Pro Pro Ala Gly
   1815              1820              1825

gcc act ggc ttt gtt gtc agt ggc ctg gtg gga gct gct gtt ggc agc   5875
Ala Thr Gly Phe Val Val Ser Gly Leu Val Gly Ala Ala Val Gly Ser
1830              1835              1840              1845

ata ggc ttg ggt aaa gtg ctg gtg gac atc ctg gca ggg tat ggt gcg   5923
Ile Gly Leu Gly Lys Val Leu Val Asp Ile Leu Ala Gly Tyr Gly Ala
             1850              1855              1860

ggc att tcg ggg gcc ctc gtc gcg ttt aag atc atg tct ggc gag aag   5971
Gly Ile Ser Gly Ala Leu Val Ala Phe Lys Ile Met Ser Gly Glu Lys
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          1865                1870                1875

ccc tcc atg gag gat gtc atc aac ttg ctg cct ggg att ctg tct cca   6019
Pro Ser Met Glu Asp Val Ile Asn Leu Leu Pro Gly Ile Leu Ser Pro
      1880                1885                1890

ggt gct ctg gtg gtg gga gtc atc tgc gcg gcc att ctg cgc cgc cat   6067
Gly Ala Leu Val Val Gly Val Ile Cys Ala Ala Ile Leu Arg Arg His
   1895                1900                1905

gtg gga ccg ggg gaa ggc gcg gtc caa tgg atg aac agg ctt atc gcc   6115
Val Gly Pro Gly Glu Gly Ala Val Gln Trp Met Asn Arg Leu Ile Ala
1910                1915                1920                1925

ttc gct tcc aga gga aac cac gtc gcc cct act cac tac gtg acg gag   6163
Phe Ala Ser Arg Gly Asn His Val Ala Pro Thr His Tyr Val Thr Glu
             1930                1935                1940

tcg gat gcg tcg cag cgt gtc acc caa ctg ctt ggc tct ctc act ata   6211
Ser Asp Ala Ser Gln Arg Val Thr Gln Leu Leu Gly Ser Leu Thr Ile
          1945                1950                1955

act agt cta ctc agg aga ctt cac aac tgg atc act gag gat tgc ccc   6259
Thr Ser Leu Leu Arg Arg Leu His Asn Trp Ile Thr Glu Asp Cys Pro
      1960                1965                1970

atc cca tgc gcc ggc tcg tgg ctc cgc gat gtg tgg gac tgg gtc tgt   6307
Ile Pro Cys Ala Gly Ser Trp Leu Arg Asp Val Trp Asp Trp Val Cys
   1975                1980                1985

acc atc cta aca gac ttt aag aac tgg ctg acc tcc aag ctg ttc cca   6355
Thr Ile Leu Thr Asp Phe Lys Asn Trp Leu Thr Ser Lys Leu Phe Pro
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1990                1995                2000                2005

aag atg cct ggc ctc ccc ttt atc tct tgc caa aag ggg tac aag ggc   6403
Lys Met Pro Gly Leu Pro Phe Ile Ser Cys Gln Lys Gly Tyr Lys Gly
                2010                2015                2020

gtg tgg gcc ggc act ggc atc atg acc aca cga tgc ccc tgc ggc gcc   6451
Val Trp Ala Gly Thr Gly Ile Met Thr Thr Arg Cys Pro Cys Gly Ala
            2025                2030                2035

aac atc tct ggc aac gtc cgc ttg ggc tct atg aga atc aca gga ccc   6499
Asn Ile Ser Gly Asn Val Arg Leu Gly Ser Met Arg Ile Thr Gly Pro
        2040                2045                2050

aaa acc tgc atg aac acc tgg cag ggg acc ttt cct atc aat tgt tat   6547
Lys Thr Cys Met Asn Thr Trp Gln Gly Thr Phe Pro Ile Asn Cys Tyr
    2055                2060                2065

aca gaa ggc cag tgc ttg ccg aaa ccc gcg tta aac ttc aag acc gcc   6595
Thr Glu Gly Gln Cys Leu Pro Lys Pro Ala Leu Asn Phe Lys Thr Ala
2070                2075                2080                2085

atc tgg aga gtg gcg gcc tca gag tac gcg gaa gtg acg cag cac gga   6643
Ile Trp Arg Val Ala Ala Ser Glu Tyr Ala Glu Val Thr Gln His Gly
                2090                2095                2100

tca tat gcc tat ata aca ggg ctg acc act gac aac tta aaa gtc cct   6691
Ser Tyr Ala Tyr Ile Thr Gly Leu Thr Thr Asp Asn Leu Lys Val Pro
            2105                2110                2115

tgc caa ctc ccc tct cca gag ttt ttc tct tgg gtg gac gga gta caa   6739
Cys Gln Leu Pro Ser Pro Glu Phe Phe Ser Trp Val Asp Gly Val Gln
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      2120              2125              2130

atc cat agg tcc gcc ccc aca cca aag ccg ttt ttc cgg gat gag gtc   6787
Ile His Arg Ser Ala Pro Thr Pro Lys Pro Phe Phe Arg Asp Glu Val
   2135              2140              2145

tcg ttc agc gtt ggg ctc aat tca ttt gtc gtc ggg tct cag ctt ccc   6835
Ser Phe Ser Val Gly Leu Asn Ser Phe Val Val Gly Ser Gln Leu Pro
2150              2155              2160              2165

tgt gac cct gag ccc gac act gag gta gtg atg tcc atg cta aca gac   6883
Cys Asp Pro Glu Pro Asp Thr Glu Val Val Met Ser Met Leu Thr Asp
             2170              2175              2180

cca tcc cat atc acg gcg gag gct gca gcg cgg cgt tta gcg cgg ggg   6931
Pro Ser His Ile Thr Ala Glu Ala Ala Ala Arg Arg Leu Ala Arg Gly
         2185              2190              2195

tca ccc cca tct gag gca agc tcc tca gcg agc cag ctg tcg gcg cca   6979
Ser Pro Pro Ser Glu Ala Ser Ser Ser Ala Ser Gln Leu Ser Ala Pro
      2200              2205              2210

tcg ctg cga gcc acc tgc acc acc cac ggt agg acc tat gat gtg gac   7027
Ser Leu Arg Ala Thr Cys Thr Thr His Gly Arg Thr Tyr Asp Val Asp
   2215              2220              2225

atg gtg gat gcc aac ctg ttc atg ggg ggc ggc gtg att cgg ata gag   7075
Met Val Asp Ala Asn Leu Phe Met Gly Gly Gly Val Ile Arg Ile Glu
2230              2235              2240              2245

tct gag tcc aaa gtg gtc gtt ctg gac tcc ctc gac tca atg acc gag   7123
Ser Glu Ser Lys Val Val Val Leu Asp Ser Leu Asp Ser Met Thr Glu
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            2250              2255              2260

gaa gag ggc gac ctt gag cct tca gta cca tcg gag tat atg ctc ccc   7171
Glu Glu Gly Asp Leu Glu Pro Ser Val Pro Ser Glu Tyr Met Leu Pro
         2265              2270              2275

agg aag agg ttc cca ccg gcc tta ccg gct tgg gcg cgg cct gat tac   7219
Arg Lys Arg Phe Pro Pro Ala Leu Pro Ala Trp Ala Arg Pro Asp Tyr
      2280              2285              2290

aac cca ccg ctt gtg gaa tcg tgg aag agg cca gat tac caa cca ccc   7267
Asn Pro Pro Leu Val Glu Ser Trp Lys Arg Pro Asp Tyr Gln Pro Pro
   2295              2300              2305

act gtt gcg ggc tgt gct ctc ccc ccc ccc aaa aag acc ccg acg cct   7315
Thr Val Ala Gly Cys Ala Leu Pro Pro Pro Lys Lys Thr Pro Thr Pro
2310              2315              2320              2325

cct cca agg aga cgc cgg aca gtg ggt ctg agc gag agc acc ata gga   7363
Pro Pro Arg Arg Arg Arg Thr Val Gly Leu Ser Glu Ser Thr Ile Gly
            2330              2335              2340

gat gcc ctc caa cag ctg gcc atc aag tcc ttt ggc cag ccc ccc cca   7411
Asp Ala Leu Gln Gln Leu Ala Ile Lys Ser Phe Gly Gln Pro Pro Pro
         2345              2350              2355

agc ggc gat tca ggc ctt tcc acg ggg gcg gac gcc gcc gac tcc ggc   7459
Ser Gly Asp Ser Gly Leu Ser Thr Gly Ala Asp Ala Ala Asp Ser Gly
      2360              2365              2370

gat cgg aca ccc cct gac gag ttg gct ctt tcg gag aca ggt tct acc   7507
Asp Arg Thr Pro Pro Asp Glu Leu Ala Leu Ser Glu Thr Gly Ser Thr
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   2375              2380              2385

tcc tcc atg ccc ccc ctc gag ggg gag cct ggg gac cca gac ctg gag   7555
Ser Ser Met Pro Pro Leu Glu Gly Glu Pro Gly Asp Pro Asp Leu Glu
2390              2395              2400              2405

cct gag cag gta gag ctt caa cct cct ccc cag ggg ggg gag gca gct   7603
Pro Glu Gln Val Glu Leu Gln Pro Pro Pro Gln Gly Gly Glu Ala Ala
             2410              2415              2420

ccc ggc tcg gac tcg ggg tcc tgg tct act tgc tcc gag gag gat gac   7651
Pro Gly Ser Asp Ser Gly Ser Trp Ser Thr Cys Ser Glu Glu Asp Asp
         2425              2430              2435

tcc gtc gtg tgc tgc tcc atg tca tat tcc tgg acc ggg gct cta ata   7699
Ser Val Val Cys Cys Ser Met Ser Tyr Ser Trp Thr Gly Ala Leu Ile
      2440              2445              2450

act cct tgt agc ccc gaa gag gaa aag ttg cca att aac tcc ttg agc   7747
Thr Pro Cys Ser Pro Glu Glu Glu Lys Leu Pro Ile Asn Ser Leu Ser
   2455              2460              2465

aac tcg ctg ttg cga tac cat aac aag gta tac tgt act aca tca aag   7795
Asn Ser Leu Leu Arg Tyr His Asn Lys Val Tyr Cys Thr Thr Ser Lys
2470              2475              2480              2485

agt gcc tca cta agg gct aaa aag gta act ttt gat agg atg caa gtg   7843
Ser Ala Ser Leu Arg Ala Lys Lys Val Thr Phe Asp Arg Met Gln Val
             2490              2495              2500

ctc gac gcc tat tat gat tca gtc tta aag gac atc aag cta gcg gcc   7891
Leu Asp Ala Tyr Tyr Asp Ser Val Leu Lys Asp Ile Lys Leu Ala Ala
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          2505                2510                2515

tcc aag gtc agc gca agg ctc ctc acc tta gag gag gcg tgc caa ttg   7939
Ser Lys Val Ser Ala Arg Leu Leu Thr Leu Glu Glu Ala Cys Gln Leu
      2520                2525                2530

acc cca ccc cac tct gca aga tcc aag tat ggg ttt ggg gct aag gag   7987
Thr Pro Pro His Ser Ala Arg Ser Lys Tyr Gly Phe Gly Ala Lys Glu
   2535                2540                2545

gtc cgc agc ttg tcc ggg agg gcc gtc aac cac atc aag tcc gtg tgg   8035
Val Arg Ser Leu Ser Gly Arg Ala Val Asn His Ile Lys Ser Val Trp
2550                2555                2560                2565

aag gac ctc ttg gaa gac tca caa aca cca att cct aca acc atc atg   8083
Lys Asp Leu Leu Glu Asp Ser Gln Thr Pro Ile Pro Thr Thr Ile Met
             2570                2575                2580

gcc aaa aat gag gtg ttc tgc gtg gac ccc gcc aag ggg ggt aaa aaa   8131
Ala Lys Asn Glu Val Phe Cys Val Asp Pro Ala Lys Gly Gly Lys Lys
          2585                2590                2595

cca gct cgc ctt atc gtt tac cct gac ctc ggc gtc agg gtc tgc gag   8179
Pro Ala Arg Leu Ile Val Tyr Pro Asp Leu Gly Val Arg Val Cys Glu
      2600                2605                2610

aag atg gcc ctt tat gat gtc aca caa aag ctt cct cag gcg gtg atg   8227
Lys Met Ala Leu Tyr Asp Val Thr Gln Lys Leu Pro Gln Ala Val Met
   2615                2620                2625

ggg gct tct tat ggc ttc cag tac tcc ccc gct cag cgg gtg gag ttt   8275
Gly Ala Ser Tyr Gly Phe Gln Tyr Ser Pro Ala Gln Arg Val Glu Phe
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2630              2635              2640              2645

ctc ttg aag gca tgg gcg gaa aag aga gac cct atg ggt ttt tcg tat   8323
Leu Leu Lys Ala Trp Ala Glu Lys Arg Asp Pro Met Gly Phe Ser Tyr
             2650              2655              2660

gat acc cga tgc ttt gac tca acc gtc act gag aga gac atc agg act   8371
Asp Thr Arg Cys Phe Asp Ser Thr Val Thr Glu Arg Asp Ile Arg Thr
         2665              2670              2675

gag gag tcc ata tac cag gcc tgc tcc tta ccc gag gag gcc cga act   8419
Glu Glu Ser Ile Tyr Gln Ala Cys Ser Leu Pro Glu Glu Ala Arg Thr
     2680              2685              2690

gcc ata cac tcg ctg act gag aga ctc tat gtg gga ggg ccc atg ttc   8467
Ala Ile His Ser Leu Thr Glu Arg Leu Tyr Val Gly Gly Pro Met Phe
  2695              2700              2705

aac agc aag ggc cag tcc tgc ggg tac agg cgt tgc cgc gcc agc ggg   8515
Asn Ser Lys Gly Gln Ser Cys Gly Tyr Arg Arg Cys Arg Ala Ser Gly
2710              2715              2720              2725

gtg ctt acc act agt atg ggg aac acc atc aca tgc tat gta aaa gcc   8563
Val Leu Thr Thr Ser Met Gly Asn Thr Ile Thr Cys Tyr Val Lys Ala
             2730              2735              2740

cta gcg gct tgc aag gct gcg ggg ata att gcg ccc acg atg ctg gta   8611
Leu Ala Ala Cys Lys Ala Ala Gly Ile Ile Ala Pro Thr Met Leu Val
         2745              2750              2755

tgc ggc gac gac ttg gtc gtc atc tca gaa agc cag ggg act gag gag   8659
Cys Gly Asp Asp Leu Val Val Ile Ser Glu Ser Gln Gly Thr Glu Glu
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      2760              2765              2770

gac gag cgg aac ctg aga gcc ttc acg gag gct atg acc agg tat tct   8707
Asp Glu Arg Asn Leu Arg Ala Phe Thr Glu Ala Met Thr Arg Tyr Ser
   2775              2780              2785

gcc cct cct ggt gac ccc ccc aga ccg gaa tat gac ctg gag cta ata   8755
Ala Pro Pro Gly Asp Pro Pro Arg Pro Glu Tyr Asp Leu Glu Leu Ile
2790              2795              2800              2805

aca tct tgt tcc tca aac gtg tct gtg gca ctt ggc cca cag ggc cgc   8803
Thr Ser Cys Ser Ser Asn Val Ser Val Ala Leu Gly Pro Gln Gly Arg
             2810              2815              2820

cgc aga tac tac ctg acc aga gac ccc acc act tca att gcc cgg gct   8851
Arg Arg Tyr Tyr Leu Thr Arg Asp Pro Thr Thr Ser Ile Ala Arg Ala
         2825              2830              2835

gcc tgg gaa aca gtt aga cac tcc cct gtc aat tca tgg ctg gga aac   8899
Ala Trp Glu Thr Val Arg His Ser Pro Val Asn Ser Trp Leu Gly Asn
      2840              2845              2850

atc atc cag tac gct cca acc ata tgg gtt cgc atg gtc ctg atg aca   8947
Ile Ile Gln Tyr Ala Pro Thr Ile Trp Val Arg Met Val Leu Met Thr
   2855              2860              2865

cac ttc ttc tcc att ctc atg gcc cag gac acc cta gac cag aac ctt   8995
His Phe Phe Ser Ile Leu Met Ala Gln Asp Thr Leu Asp Gln Asn Leu
2870              2875              2880              2885

aac ttt gaa atg tac gga tcg gtg tac tcc gtg agt cct ctg gac ctc   9043
Asn Phe Glu Met Tyr Gly Ser Val Tyr Ser Val Ser Pro Leu Asp Leu
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            2890              2895              2900

cca gcc ata att gaa agg tta cac ggg ctt gac gcc ttc tct ctg cac   9091
Pro Ala Ile Ile Glu Arg Leu His Gly Leu Asp Ala Phe Ser Leu His
          2905              2910              2915

aca tac act ccc cac gaa ctg acg cgg gtg gct tca gcc ctc aga aaa   9139
Thr Tyr Thr Pro His Glu Leu Thr Arg Val Ala Ser Ala Leu Arg Lys
      2920              2925              2930

ctt ggg gcg cca ccc ctc aga gcg tgg aag agt cgg gcg cgt gca gtt   9187
Leu Gly Ala Pro Pro Leu Arg Ala Trp Lys Ser Arg Ala Arg Ala Val
   2935              2940              2945

agg gcg tcc ctc atc tcc cgt ggg ggg agg gcg gcc gtt tgc ggt cgg   9235
Arg Ala Ser Leu Ile Ser Arg Gly Gly Arg Ala Ala Val Cys Gly Arg
2950              2955              2960              2965

tac ctc ttc aac tgg gcg gtg aag acc aag ctc aaa ctc act cct ttg   9283
Tyr Leu Phe Asn Trp Ala Val Lys Thr Lys Leu Lys Leu Thr Pro Leu
             2970              2975              2980

ccg gag gca cgc ctc ctg gat ttg tcc agt tgg ttt acc gtc ggc gcc   9331
Pro Glu Ala Arg Leu Leu Asp Leu Ser Ser Trp Phe Thr Val Gly Ala
          2985              2990              2995

ggc ggg ggc gac att tat cac agc gtg tcg cgt gcc cga ccc cgc cta   9379
Gly Gly Gly Asp Ile Tyr His Ser Val Ser Arg Ala Arg Pro Arg Leu
      3000              3005              3010

tta ctc ctt agc cta ctc cta ctt tct gta ggg gta ggc ctc ttc cta   9427
Leu Leu Leu Ser Leu Leu Leu Leu Ser Val Gly Val Gly Leu Phe Leu
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3015 3020 3025

ctc ccc gct cga tag agcggcacac attagctaca ctccatagct aactgttcct 9482

Leu Pro Ala Arg

3030

ttttttttt ttttttttt ttttttttt ttttttcctt ttttttttt tttccctctt 9542

tcttccctc tcatcttatt ctactttctt tcttggtggc tccatcttag ccctggtcac 9602

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tgcagatcat gt 9674

<210> 6

<211> 3033

<212> PRT

<213> Hepatitis C virus

<400> 6

Met Ser Thr Asn Pro Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn

1 5 10 15

Arg Arg Pro Gln Asp Val Lys Phe Pro Gly Gly Gly Gln Ile Val Gly

20 25 30

Gly Val Tyr Leu Leu Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Ala

35 40 45

Thr Arg Lys Ala Ser Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro

50 55 60

Ile Pro Lys His Arg Arg Ser Thr Gly Lys Ser Trp Gly Lys Pro Gly

65 70 75 80

Tyr Pro Trp Pro Leu Tyr Gly Asn Glu Gly Leu Gly Trp Ala Gly Trp

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                85                  90                  95
Leu Leu Ser Pro Arg Gly Ser Arg Pro Ser Trp Gly Pro Asn Asp Pro
            100                 105                 110
Arg His Arg Ser Arg Asn Val Gly Lys Val Ile Asp Thr Leu Thr Cys
        115                 120                 125
Gly Phe Ala Asp Leu Leu Gly Tyr Val Pro Val Val Gly Ala Pro Leu
    130                 135                 140
Ser Gly Val Ala Ser Ala Leu Ala His Gly Val Arg Val Leu Glu Asp
145                 150                 155                 160
Gly Val Asn Phe Ala Thr Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile
                165                 170                 175
Phe Leu Leu Ala Leu Leu Ser Cys Ile Thr Thr Pro Val Ser Ala Val
            180                 185                 190
Gln Val Lys Asn Thr Ser Asn Ala Tyr Met Ala Thr Asn Asp Cys Ser
        195                 200                 205
Asn Asp Ser Ile Thr Trp Gln Leu Glu Ala Ala Val Leu His Val Pro
    210                 215                 220
Gly Cys Val Pro Cys Glu Lys Met Gly Asn Thr Ser Arg Cys Trp Ile
225                 230                 235                 240
Pro Val Ser Pro Asn Val Ala Val Arg Gln Pro Gly Ala Leu Thr Arg
                245                 250                 255
Gly Leu Arg Thr His Ile Asp Met Val Val Leu Ser Ala Thr Leu Cys
            260                 265                 270
Ser Ala Leu Tyr Val Gly Asp Leu Cys Gly Gly Val Met Leu Ala Ser
        275                 280                 285
Gln Met Phe Ile Val Ser Pro Gln His His Trp Phe Val Gln Glu Cys
    290                 295                 300
Asn Cys Ser Ile Tyr Pro Gly Ala Ile Thr Gly His Arg Met Ala Trp
305                 310                 315                 320
Asp Met Met Met Asn Trp Ser Pro Thr Thr Thr Met Ile Leu Ala Tyr
                325                 330                 335
Val Met Arg Val Pro Glu Val Ile Ile Asp Ile Ile Ser Gly Ala His
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340 345 350

Trp Gly Val Met Phe Gly Leu Ala Tyr Phe Ser Met Gln Gly Ala Trp

355 360 365

Ala Lys Val Val Val Ile Leu Leu Leu Ala Ser Gly Val Asp Ala Tyr

370 375 380

Thr Thr Thr Thr Gly Ser Ala Ala Gly Arg Thr Thr Ser Ser Leu Ala

385 390 395 400

Ser Ala Phe Ser Pro Gly Ala Arg Gln Asn Ile Gln Leu Ile Asn Thr

405 410 415

Asn Gly Ser Trp His Ile Asn Arg Thr Ala Leu Asn Cys Asn Asp Ser

420 425 430

Leu His Thr Gly Phe Phe Thr Ala Leu Phe Tyr Ile His Lys Phe Asn

435 440 445

Ser Ser Gly Cys Pro Glu Arg Leu Ser Ala Cys Arg Asn Ile Glu Asp

450 455 460

Phe Arg Ile Gly Trp Gly Ala Leu Gln Tyr Asp Asp Asn Val Thr Asn

465 470 475 480

Pro Glu Asp Met Arg Pro Tyr Cys Trp His Tyr Pro Pro Lys Gln Cys

485 490 495

Gly Val Val Pro Ala Gly Thr Val Cys Gly Pro Val Tyr Cys Phe Thr

500 505 510

Pro Ser Pro Val Val Val Gly Thr Thr Asp Arg Leu Gly Val Pro Thr

515 520 525

Tyr Thr Trp Gly Glu Asn Glu Thr Asp Val Phe Leu Leu Asn Ser Thr

530 535 540

Arg Pro Pro Ser Gly Ser Trp Phe Gly Cys Thr Trp Met Asn Ser Thr

545 550 555 560

Gly Phe Thr Lys Thr Cys Gly Ala Pro Pro Cys Arg Thr Arg Ala Asp

565 570 575

Phe Asn Thr Ser Thr Asp Leu Leu Cys Pro Thr Asp Cys Phe Arg Lys

580 585 590

His Pro Glu Ala Thr Tyr Ile Lys Cys Gly Ser Gly Pro Trp Leu Thr

595 600 605

Pro Lys Cys Leu Val Asp Tyr Pro Tyr Arg Leu Trp His Tyr Pro Cys

610 615 620

Thr Val Asn Tyr Ser Thr Phe Lys Ile Arg Met Tyr Val Gly Gly Val

625 630 635 640

Glu His Arg Leu Met Ala Ala Cys Asn Phe Thr Arg Gly Asp Arg Cys

645 650 655

Asn Leu Glu Asp Arg Asp Arg Ser Gln Gln Thr Pro Leu Leu His Ser

660 665 670

Thr Thr Glu Trp Ala Ile Leu Pro Cys Ser Phe Ser Asp Leu Pro Ala

675 680 685

Leu Ser Thr Gly Leu Leu His Leu His Gln Asn Ile Val Asp Val Gln

690 695 700

Tyr Met Tyr Gly Leu Ser Pro Ala Leu Thr Gln Tyr Ile Val Arg Trp

705 710 715 720

Glu Trp Val Val Leu Leu Phe Leu Leu Leu Ala Asp Ala Arg Val Cys

725 730 735

Ala Cys Leu Trp Met Leu Ile Leu Leu Gly Gln Ala Glu Ala Ala Leu

740 745 750

Glu Lys Leu Val Val Leu His Ala Ala Ser Ala Ala Ser Cys Asn Gly

755 760 765

Phe Leu Tyr Phe Val Ile Phe Leu Val Ala Ala Trp His Ile Lys Gly

770 775 780

Arg Val Val Pro Leu Ala Ala Tyr Ser Leu Thr Gly Leu Trp Pro Phe

785 790 795 800

Cys Leu Leu Leu Leu Ala Leu Pro Gln Gln Ala Tyr Ala Tyr Asp Ala

805 810 815

Ser Val His Gly Gln Val Gly Ala Ala Leu Leu Val Leu Ile Thr Leu

820 825 830

Phe Thr Leu Thr Pro Gly Tyr Lys Thr Leu Leu Ser Gln Ser Leu Trp

835 840 845

Trp Leu Cys Tyr Leu Leu Thr Leu Ala Glu Thr Met Val Gln Glu Trp

850 855 860

Ala Pro Ser Met Gln Ala Arg Gly Gly Arg Asp Gly Ile Ile Trp Ala

865 870 875 880

Ala Thr Ile Phe Cys Pro Gly Val Val Phe Asp Ile Thr Lys Trp Leu

885 890 895

Leu Ala Val Leu Gly Pro Gly Tyr Leu Leu Arg Gly Ala Leu Thr Arg

900 905 910

Val Pro Tyr Phe Val Arg Ala His Ala Leu Leu Arg Met Cys Thr Met

915 920 925

Val Arg His Leu Ala Gly Gly Arg Tyr Val Gln Met Ala Leu Leu Ala

930 935 940

Leu Gly Arg Trp Thr Gly Thr Tyr Ile Tyr Asp His Leu Thr Pro Met

945 950 955 960

Ser Asp Trp Ala Ala Ser Gly Leu Arg Asp Leu Ala Val Ala Val Glu

965 970 975

Pro Ile Ile Phe Ser Pro Met Glu Lys Lys Val Ile Val Trp Gly Ala

980 985 990

Glu Thr Ala Ala Cys Gly Asp Ile Leu His Gly Leu Pro Val Ser Ala

995 1000 1005

Arg Leu Gly Arg Glu Ile Leu Leu Gly Pro Ala Asp Gly Tyr Thr Ser

1010 1015 1020

Lys Gly Trp Lys Leu Leu Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr

1025 1030 1035 1040

Arg Gly Leu Leu Gly Ser Ile Val Val Ser Met Thr Gly Arg Asp Lys

1045 1050 1055

Thr Glu Gln Ala Gly Glu Val Gln Val Leu Ser Thr Val Thr Gln Ser

1060 1065 1070

Phe Leu Gly Thr Ser Ile Ser Gly Val Leu Trp Thr Val Tyr His Gly

1075 1080 1085

Ala Gly Asn Lys Thr Leu Ala Gly Ser Arg Gly Pro Val Thr Gln Met

1090 1095 1100

Tyr Ser Ser Ala Glu Gly Asp Leu Val Gly Trp Pro Ser Pro Pro Gly

1105 1110 1115 1120

Thr Lys Ser Leu Glu Pro Cys Thr Cys Gly Ala Val Asp Leu Tyr Leu

1125 1130 1135

Val Thr Arg Asn Ala Asp Val Ile Pro Ala Arg Arg Arg Gly Asp Lys

1140 1145 1150

Arg Gly Ala Leu Leu Ser Pro Arg Pro Leu Ser Thr Leu Lys Gly Ser

1155 1160 1165

Ser Gly Gly Pro Val Leu Cys Pro Arg Gly His Ala Val Gly Ile Phe

1170 1175 1180

Arg Ala Ala Val Cys Ser Arg Gly Val Ala Lys Ser Ile Asp Phe Ile

1185 1190 1195 1200

Pro Val Glu Thr Leu Asp Ile Val Thr Arg Ser Pro Thr Phe Ser Asp

1205 1210 1215

Asn Ser Thr Pro Pro Ala Val Pro Gln Thr Tyr Gln Val Gly Tyr Leu

1220 1225 1230

His Ala Pro Thr Gly Ser Gly Lys Ser Thr Lys Val Pro Val Ala Tyr

1235 1240 1245

Ala Ala Gln Gly Tyr Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala

1250 1255 1260

Thr Leu Gly Phe Gly Ala Tyr Leu Ser Lys Ala His Gly Ile Asn Pro

1265 1270 1275 1280

Asn Ile Arg Thr Gly Val Arg Thr Val Thr Thr Gly Glu Pro Ile Thr

1285 1290 1295

Tyr Ser Thr Tyr Gly Lys Phe Leu Ala Asp Gly Gly Cys Ala Gly Gly

1300 1305 1310

Ala Tyr Asp Ile Ile Ile Cys Asp Glu Cys His Ser Val Asp Ala Thr

1315 1320 1325

Thr Ile Leu Gly Ile Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly

1330 1335 1340

Val Arg Leu Thr Val Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr

1345 1350 1355 1360

Thr Pro His Pro Asn Ile Glu Glu Val Ala Leu Gly Gln Glu Gly Glu

1365 1370 1375

Ile Pro Phe Tyr Gly Arg Ala Phe Pro Leu Ser Tyr Ile Lys Gly Gly

1380 1385 1390

Arg His Leu Ile Phe Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala

1395 1400 1405

Thr Ala Leu Arg Gly Met Gly Leu Asn Ala Val Ala Tyr Tyr Arg Gly

1410 1415 1420

Leu Asp Val Ser Ile Ile Pro Thr Gln Gly Asp Val Val Val Val Ala

1425 1430 1435 1440

Thr Asp Ala Leu Met Thr Gly Tyr Thr Gly Asp Phe Asp Ser Val Ile

1445 1450 1455

Asp Cys Asn Val Ala Val Thr Gln Ala Val Asp Phe Ser Leu Asp Pro

1460 1465 1470

Thr Phe Thr Ile Thr Thr Gln Thr Val Pro Gln Asp Ala Val Ser Arg

1475 1480 1485

Ser Gln Arg Arg Gly Arg Thr Gly Arg Gly Arg Leu Gly Ile Tyr Arg

1490 1495 1500

Tyr Val Ser Thr Gly Glu Arg Ala Ser Gly Met Phe Asp Ser Val Val

1505 1510 1515 1520

Leu Cys Glu Cys Tyr Asp Ala Gly Ala Ala Trp Tyr Glu Leu Ser Pro

1525 1530 1535

Val Glu Thr Thr Val Arg Leu Arg Ala Tyr Phe Asn Thr Pro Gly Leu

1540 1545 1550

Pro Val Cys Gln Asp His Leu Glu Phe Trp Glu Ala Val Phe Thr Gly

1555 1560 1565

Leu Thr His Ile Asp Ala His Phe Leu Ser Gln Thr Lys Gln Ser Gly

1570 1575 1580

Glu Asn Phe Ala Tyr Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg

1585 1590 1595 1600

Ala Lys Ala Pro Pro Pro Ser Trp Asp Val Met Trp Lys Cys Leu Thr

1605 1610 1615

Arg Leu Lys Pro Thr Leu Val Gly Pro Thr Pro Leu Leu Tyr Arg Leu

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         1620                1625                1630
Gly Ser Val Thr Asn Glu Val Thr Leu Thr His Pro Val Thr Lys Tyr
      1635                1640                1645
Ile Ala Thr Cys Met Gln Ala Asp Leu Glu Val Met Thr Ser Thr Trp
   1650                1655                1660
Val Leu Ala Gly Gly Val Leu Ala Ala Val Ala Ala Tyr Cys Leu Ala
1665                1670                1675                1680
Thr Gly Cys Val Ser Ile Ile Gly Arg Leu His Ile Asn Gln Arg Ala
             1685                1690                1695
Val Val Ala Pro Asp Lys Glu Val Leu Tyr Glu Ala Phe Asp Glu Met
          1700                1705                1710
Glu Glu Cys Ala Ser Arg Ala Ala Leu Leu Glu Glu Gly Gln Arg Ile
      1715                1720                1725
Ala Glu Met Leu Lys Ser Lys Ile Gln Gly Leu Leu Gln Gln Ala Ser
   1730                1735                1740
Lys Gln Ala Gln Asp Ile Gln Pro Ala Val Gln Ala Ser Trp Pro Lys
1745                1750                1755                1760
Met Glu Gln Phe Trp Ala Lys His Met Trp Asn Phe Ile Ser Gly Ile
             1765                1770                1775
Gln Tyr Leu Ala Gly Leu Ser Thr Leu Pro Gly Asn Pro Ala Val Ala
          1780                1785                1790
Ser Met Met Ala Phe Ser Ala Ala Leu Thr Ser Pro Leu Ser Thr Ser
      1795                1800                1805
Thr Thr Ile Leu Leu Asn Ile Leu Gly Gly Trp Leu Ala Ser Gln Ile
   1810                1815                1820
Ala Pro Pro Ala Gly Ala Thr Gly Phe Val Val Ser Gly Leu Val Gly
1825                1830                1835                1840
Ala Ala Val Gly Ser Ile Gly Leu Gly Lys Val Leu Val Asp Ile Leu
             1845                1850                1855
Ala Gly Tyr Gly Ala Gly Ile Ser Gly Ala Leu Val Ala Phe Lys Ile
          1860                1865                1870
Met Ser Gly Glu Lys Pro Ser Met Glu Asp Val Ile Asn Leu Leu Pro
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      1875                1880                1885
Gly Ile Leu Ser Pro Gly Ala Leu Val Val Gly Val Ile Cys Ala Ala
   1890                1895                1900
Ile Leu Arg Arg His Val Gly Pro Gly Glu Gly Ala Val Gln Trp Met
1905                1910                1915                1920
Asn Arg Leu Ile Ala Phe Ala Ser Arg Gly Asn His Val Ala Pro Thr
             1925                1930                1935
His Tyr Val Thr Glu Ser Asp Ala Ser Gln Arg Val Thr Gln Leu Leu
          1940                1945                1950
Gly Ser Leu Thr Ile Thr Ser Leu Leu Arg Arg Leu His Asn Trp Ile
      1955                1960                1965
Thr Glu Asp Cys Pro Ile Pro Cys Ala Gly Ser Trp Leu Arg Asp Val
   1970                1975                1980
Trp Asp Trp Val Cys Thr Ile Leu Thr Asp Phe Lys Asn Trp Leu Thr
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Ser Lys Leu Phe Pro Lys Met Pro Gly Leu Pro Phe Ile Ser Cys Gln
             2005                2010                2015
Lys Gly Tyr Lys Gly Val Trp Ala Gly Thr Gly Ile Met Thr Thr Arg
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Cys Pro Cys Gly Ala Asn Ile Ser Gly Asn Val Arg Leu Gly Ser Met
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Arg Ile Thr Gly Pro Lys Thr Cys Met Asn Thr Trp Gln Gly Thr Phe
   2050                2055                2060
Pro Ile Asn Cys Tyr Thr Glu Gly Gln Cys Leu Pro Lys Pro Ala Leu
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             2085                2090                2095
Val Thr Gln His Gly Ser Tyr Ala Tyr Ile Thr Gly Leu Thr Thr Asp
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Val Asp Gly Val Gln Ile His Arg Ser Ala Pro Thr Pro Lys Pro Phe
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Gln Leu Ser Ala Pro Ser Leu Arg Ala Thr Cys Thr Thr His Gly Arg

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2260 2265 2270

Glu Tyr Met Leu Pro Arg Lys Arg Phe Pro Pro Ala Leu Pro Ala Trp

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[Sequence Listing Free Text]

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SEQ ID NOS: 9 to 12 set forth the sequences of synthetic RNAs.

SEQ ID NOS: 13 to 28 set forth the sequences of synthetic DNAs.

[Brief Description of Drawings]

[Fig. 1]

Fig. 1 is a schematic view showing procedures for constructing a template DNA for preparing the HCV-RNA replicon according to the present invention. The upper section of Fig. 1 shows the structure of the region within pJFH1 or pJCH1, with the viral genome inserted into it. The lower section of Fig. 1 shows the structure of the region within plasmid DNA pSGREP-JFH1 or pSGREP-JCH1,

with the viral genome inserted into it, that had been constructed by substituting a part of viral genome-inserted region of pJFH1 or pJCH1 with a DNA fragment containing a neomycin resistance gene and EMCV IRES. Symbols in Fig. 1 are as described below. T7, T7 RNA promoter; G, dGTP that was inserted upstream of the 5' end of the inserted DNA derived from JFH-1 or JCH-1 and downstream of the 3' end of T7 RNA promoter sequence; 5' NTR, 5' untranslated region; Core, core protein; and 3' NTR, 3' untranslated region. E1 and E2 represent envelope proteins. NS2, NS3, NS4A, NS4B, NS5A and NS5B represent non-structural proteins. Age I, Cla I and Xba I represent cleavage sites of restriction enzymes Age I, Cla I and Xba I, respectively. GDD, the position of amino acid motif GDD corresponding to the active center of NS5B protein; neo, neomycin resistance gene; and EMCV IRES, internal ribosome entry site of encephalomyocarditis virus (EMCV IRES).

[Fig. 2A]

Fig. 2 shows the nucleotide sequence of rSGREP-JFH1.

[Fig. 2B]

Fig. 2 shows the nucleotide sequence of rSGREP-JFH1.

[Fig. 2C]

Fig. 2 shows the nucleotide sequence of rSGREP-JFH1.

[Fig. 2D]

Fig. 2 shows the nucleotide sequence of rSGREP-JFH1.

[Fig. 2E]

Fig. 2 shows the nucleotide sequence of rSGREP-JFH1.

[Fig. 2F]

Fig. 2 shows the nucleotide sequence of rSGREP-JFH1.

[Fig. 3A]

Fig. 3 shows the nucleotide sequence of rSGREP-JCH1.

[Fig. 3B]

Fig. 3 shows the nucleotide sequence of rSGREP-JCH1.

[Fig. 3C]

Fig. 3 shows the nucleotide sequence of rSGREP-JCH1.

[Fig. 3D]

Fig. 3 shows the nucleotide sequence of rSGREP-JCH1.

[Fig. 3E]

Fig. 3 shows the nucleotide sequence of rSGREP-JCH1.

[Fig. 3F]

Fig. 3 shows the nucleotide sequence of rSGREP-JCH1.

[Fig. 4]

Fig. 4 shows photographs showing the colony formation of Huh7 cells to which rSGREP-JFH1, rSGREP-JFH1/GND and rSGREP-JFH1/dGDD was transfected, respectively. The amount of each of three transfected RNAs in the upper section was 100 ng and that of three transfected RNAs in the lower section was 300 ng.

[Fig. 5]

Fig. 5 shows photographs showing colony formation of Huh7 cells to which rSGREP-JFH1 and rSGREP-JCH1 respectively had been transfected when the concentration of G418 was 0.5 mg/ml of the medium. The amount of each of these RNAs transfected was 100 ng.

[Fig. 6]

Fig. 6 shows photographs showing the effect of Mung Bean Nuclease treatment conducted on the colony-forming ability of the transfected cells. The amount of rSGREP-JFH1 RNA transfected was 100 ng for both cases. The concentration of G418 was 1.0 mg/ml in both media.

[Fig. 7]

Fig. 7 shows photographs showing colony formation when total cellular RNA derived from the replicon-replicating cell clone, which had been established by transfection of rSGREP-JFH1, was retransfected to another Huh7 cells. The photograph on the left shows that the formation of 96 colonies was observed as a result, when using the total cellular RNA derived from the replicon-replicating cell

clone No. 6. The photograph on the right shows that the formation of 77 colonies was observed as a result, when using the total cellular RNA derived from the pool clones. In both cases, RNA was retransfected in an amount containing 1×10^7 copies of the replicon RNA.

[Fig. 8]

Fig. 8 shows photographs showing the results of detecting by the Northern blot method using an rSGREP-JFH1-specific probe for the total RNA derived from a cell clone that had been obtained by retransfecting the total cellular RNA (derived from the replicon-replicating cell clone established by transfection of rSGREP-JFH1) into another Huh7 cells. Explanation of the lanes is as follows. 10^8 represents sample prepared by adding 10^8 copies of the replicon RNA synthesized in vitro to total RNA extracted from Huh7 cells. 10^7 represents sample prepared by adding 10^7 copies of the replicon RNA synthesized in vitro to total RNA extracted from Huh7 cells. Huh7, total RNA extracted from untransfected Huh7 cells; pool clone, total RNA extracted from the pool clones; and 1-11, total RNA extracted from each of cell clones Nos. 1 to 11. "Replicon RNA" represents the electrophoresed position of a molecular weight marker indicating the size of rSGREP-JFH1, "28S" represents the same of a ribosomal RNA marker indicating the size of molecular weight of 4.5 kb, and "18S" represents the same of a ribosomal RNA marker indicating the size of molecular weight of 1.9 kb.

[Fig. 9]

Fig. 9 shows photographs showing the presence or the absence of the incorporation of a neomycin resistance gene into the genomic DNA of a host cell in the cell clone to which rSGREP-JFH1- or rSGREP-JCH1-derived replicated replicon RNA was retransfected. Explanation of the lanes in the photograph on the left is as follows. M, DNA molecular weight marker; 1-8, rSGREP-JFH1-derived cell clones Nos. 1 to 8; N, untransfected Huh7 cells; and P, positive control (PCR amplification product of the neomycin resistance gene). Furthermore, explanation of the lanes in the photograph on the right is as follows.

M, DNA molecular weight marker; and 1-6, rSGREP-JCH1-derived cell clones Nos. 1 to 6.

[Fig. 10]

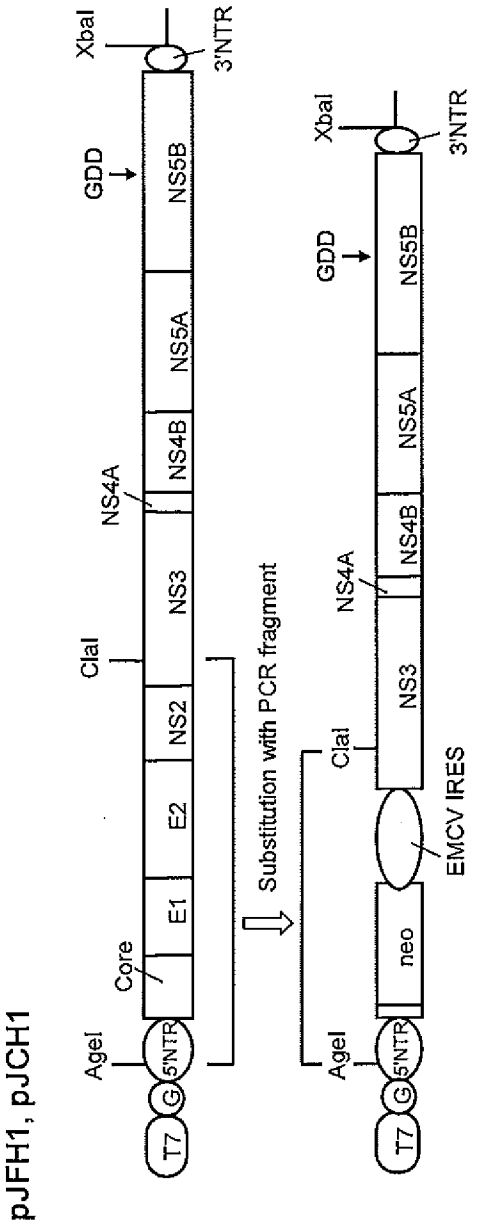
Fig. 10 shows photographs showing the results of detecting NS3 protein expressed in the cell clone that was retransfected with rSGREP-JFH1- or rSGREP-JCH1-derived replicated replicon RNA. Lanes 1 to 8 of the photograph on the left represent rSGREP-JFH1-derived cell clones Nos. 1 to 8. Lanes 1-6 of the photograph on the right represent rSGREP-JCH1-derived cell clones Nos. 1 to 6. Lane P of the photograph on the right represents NS3 protein (positive control) and N represents protein extracted from untransfected Huh7 cells (negative control).

[Fig. 11]

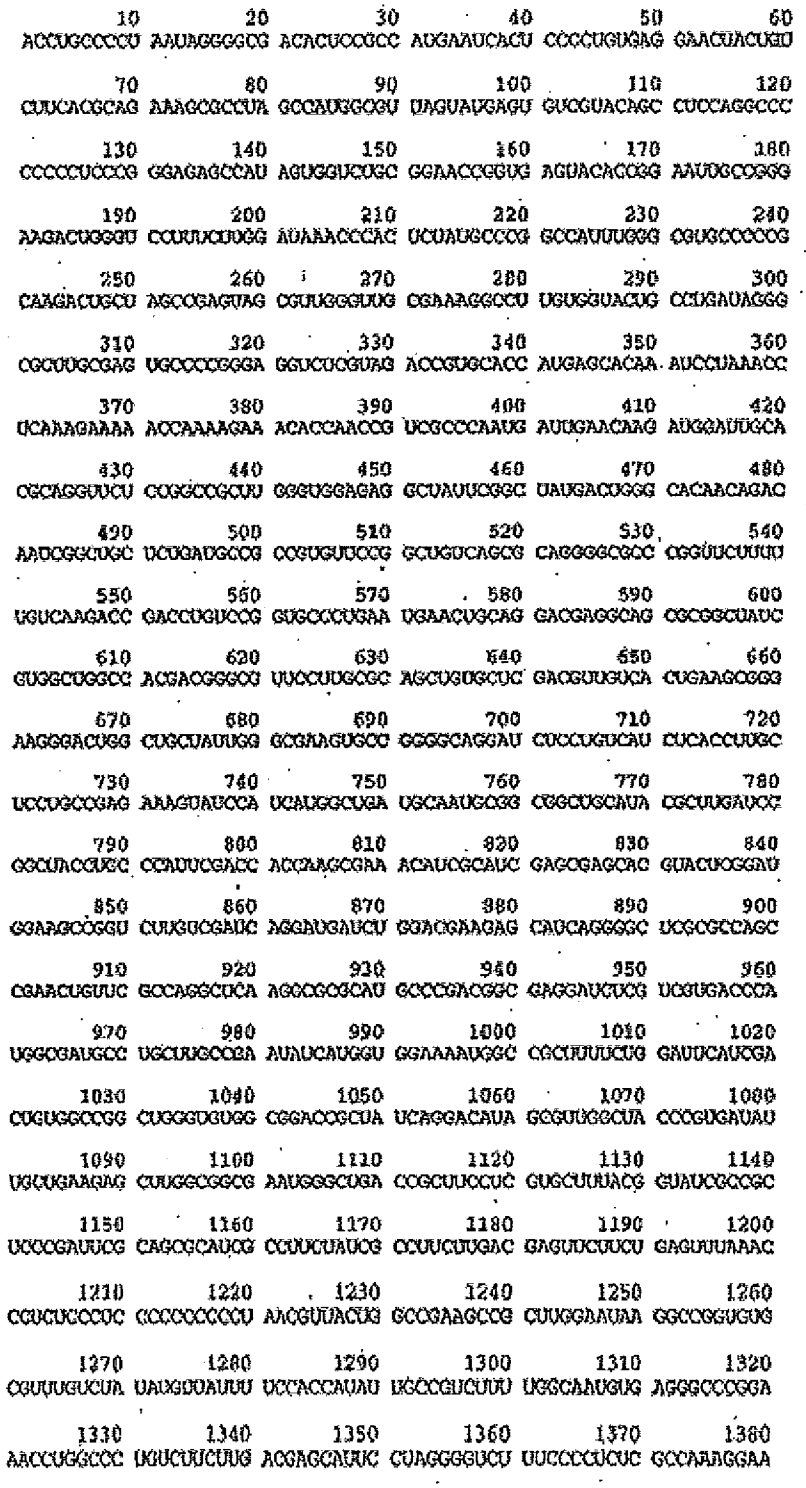
Fig. 11 shows the positions of nucleotide mutations in replicon RNAs obtained from 21 cell clones that were established through the re-transfection of rSGREP-JFH1-derived replicated replicon RNA into Huh7 cells. Mutation positions are indicated using bar lines shown with nucleotide numbers listed in Table 4. A thick bar line denotes nonsynonymous substitution and a thin bar line denotes synonymous substitution.

[Title of Document] Drawings

[Figure 1]



[Figure 2A]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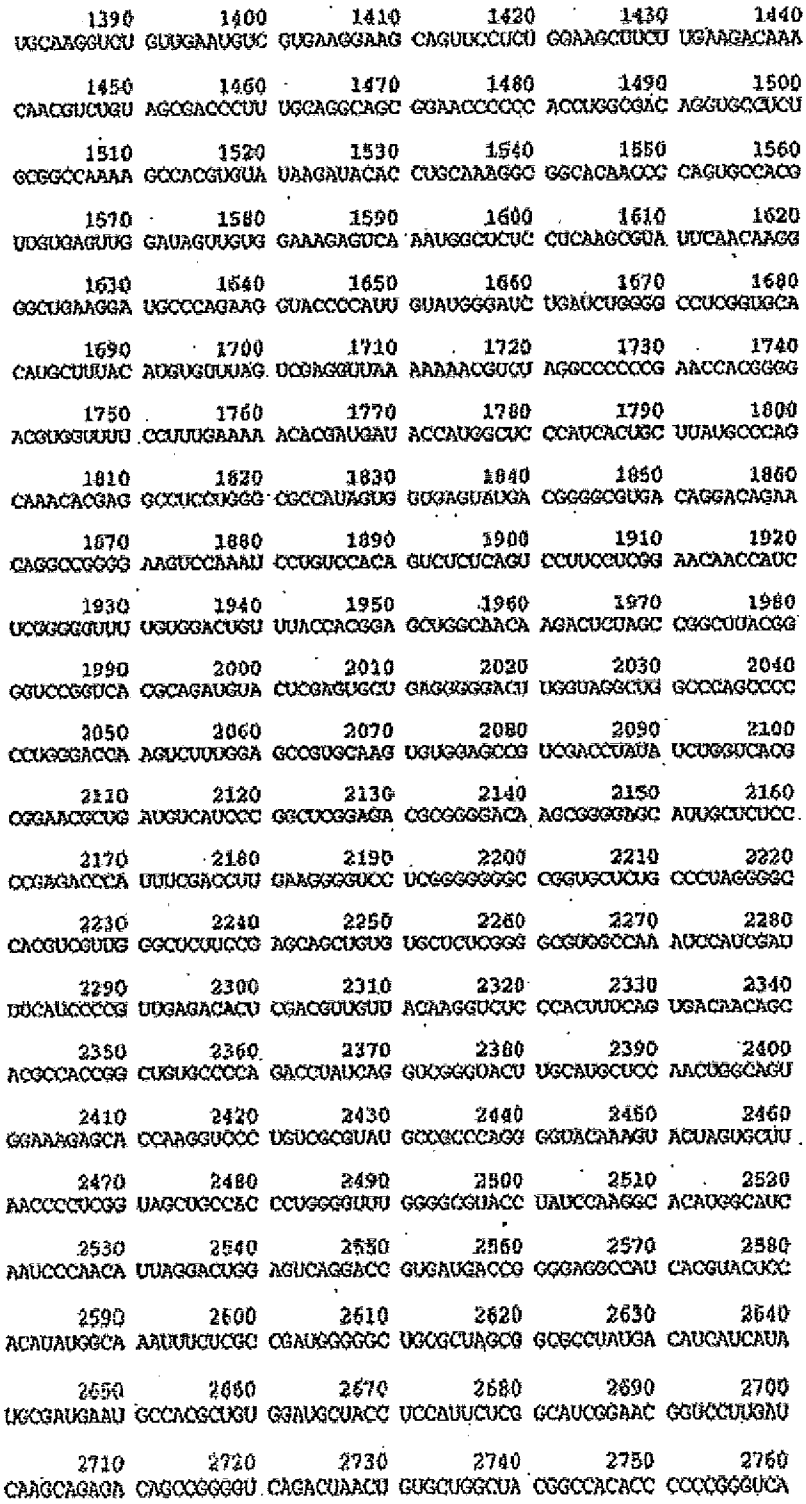
[Figure 2B]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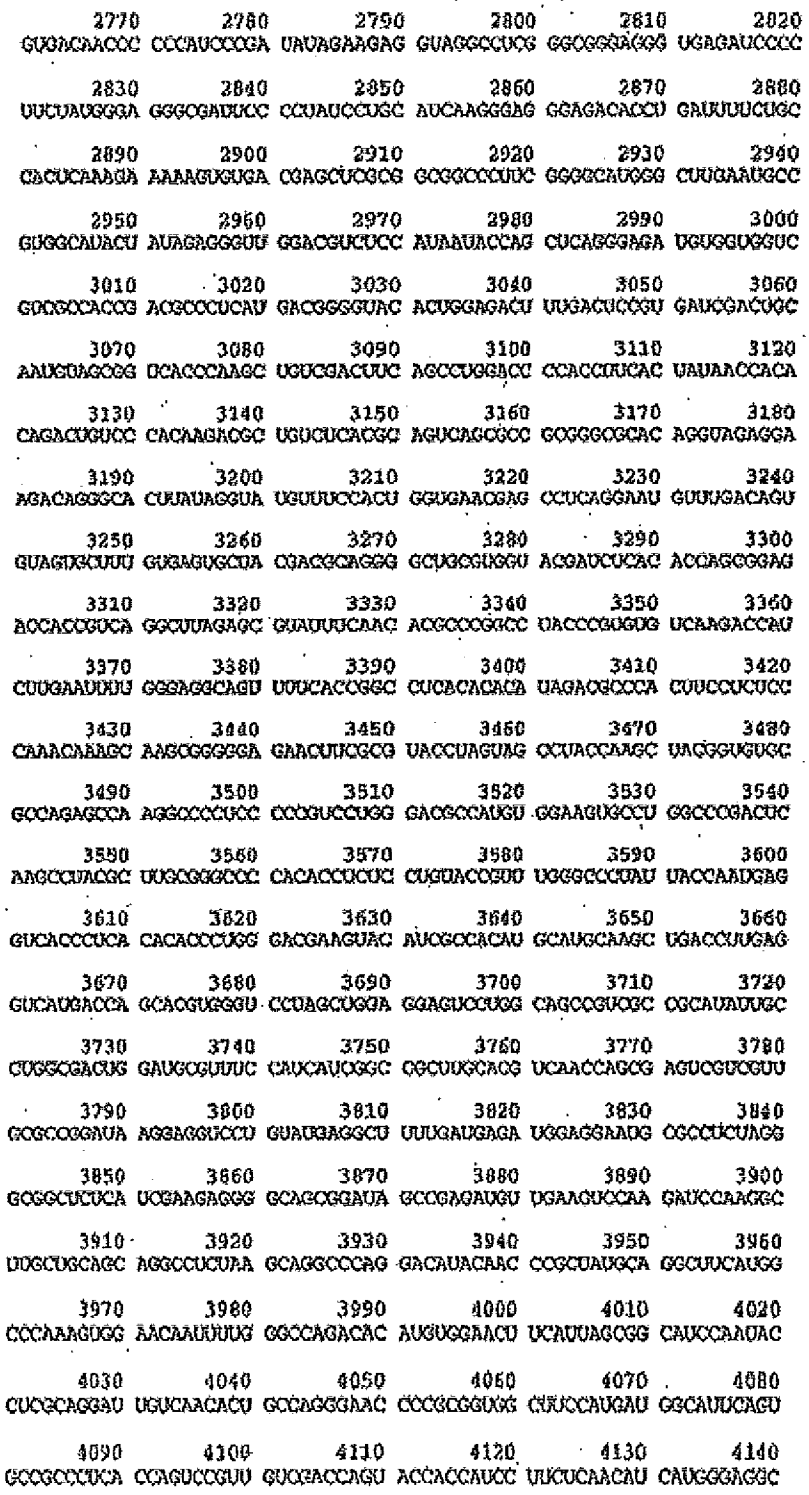
[Figure 2C]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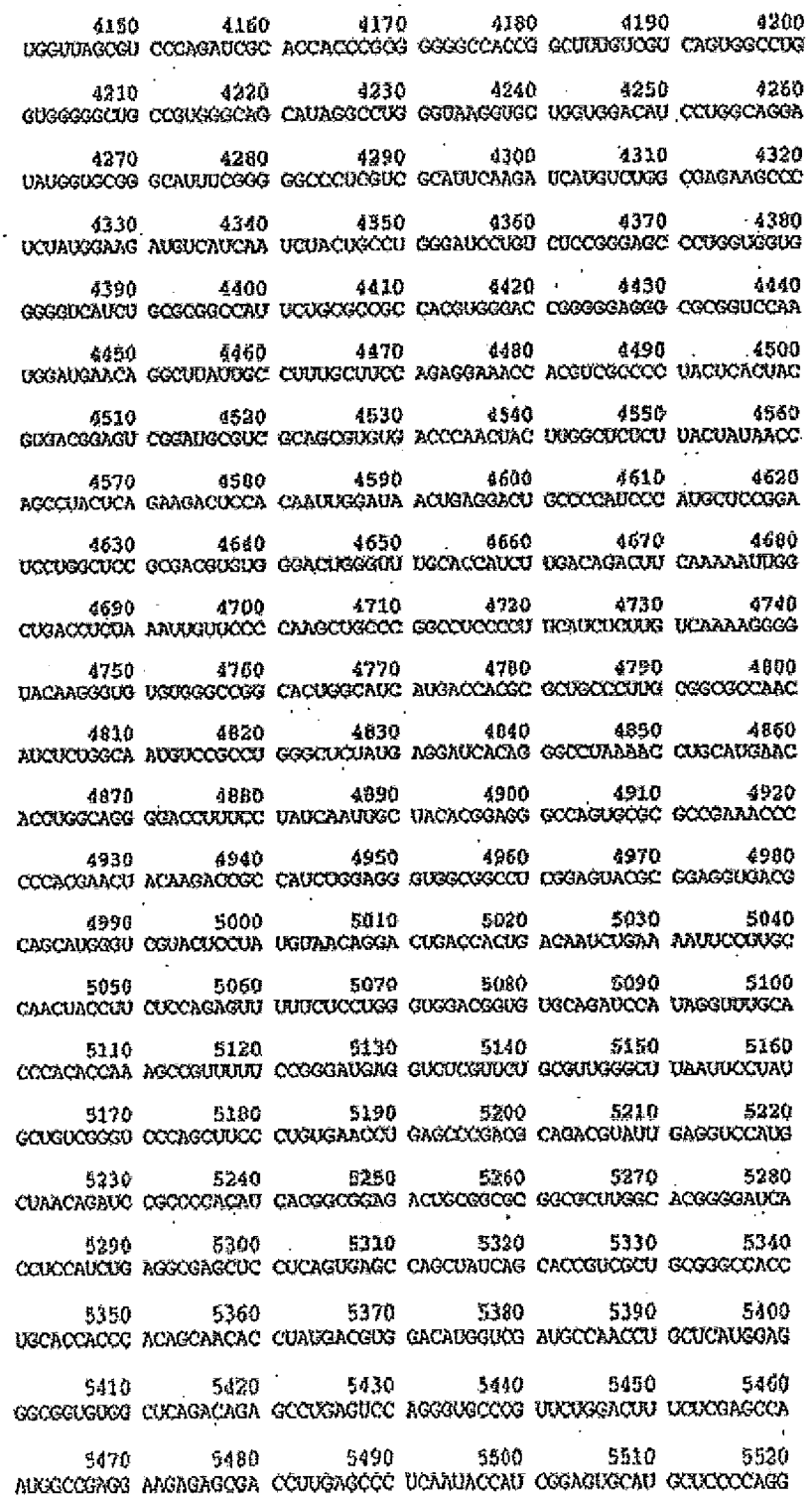
[Figure 2D]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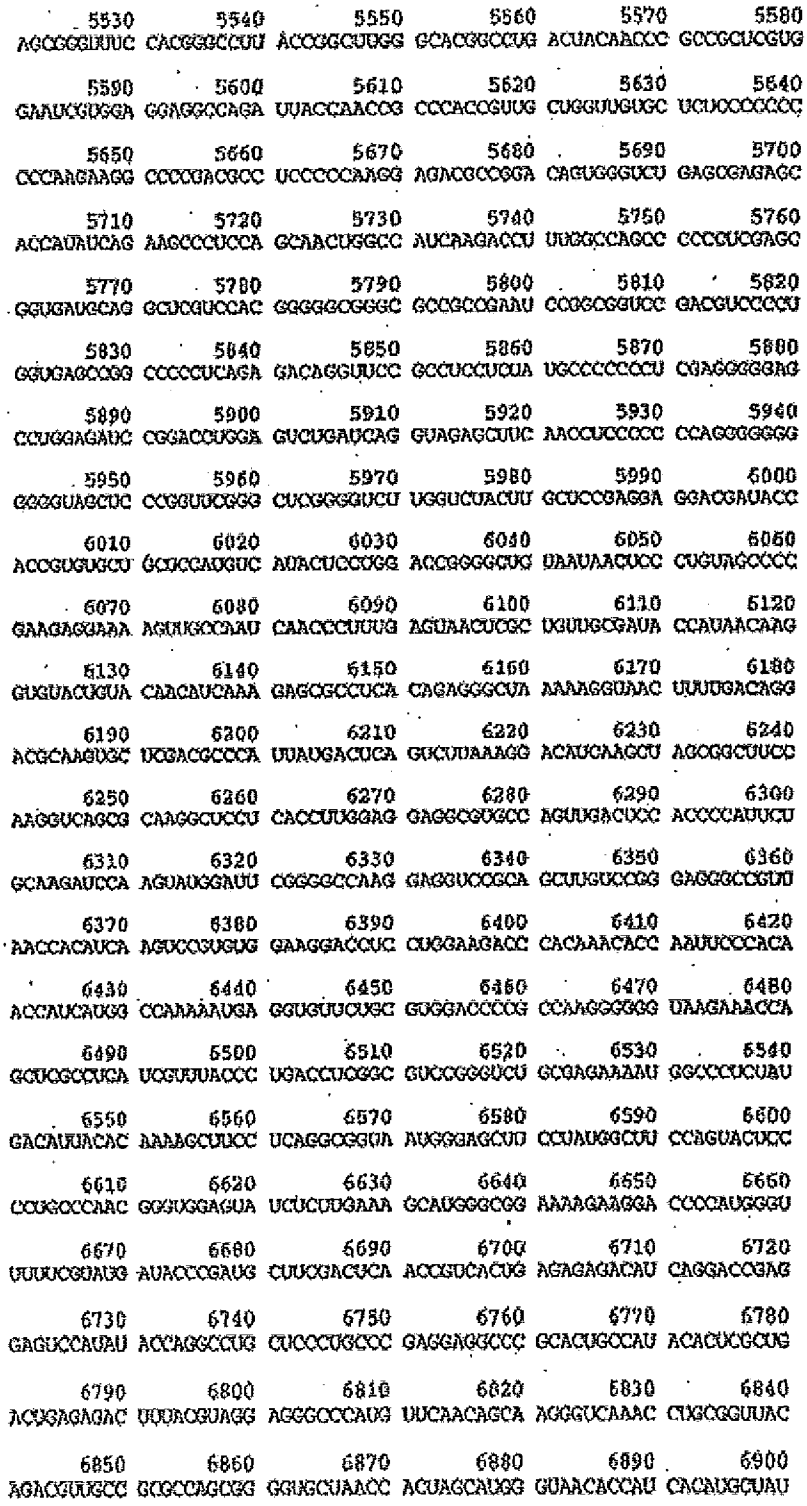
[Figure 2E]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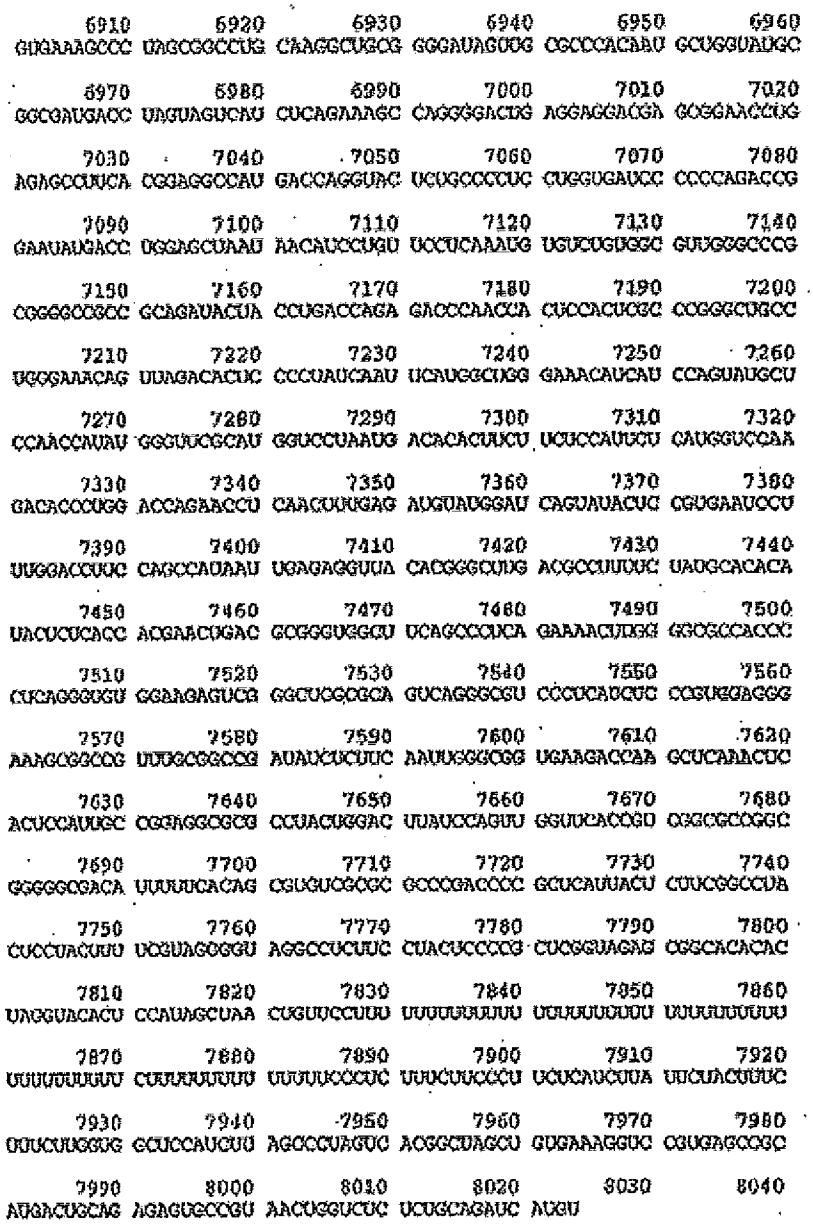
[Figure 2F]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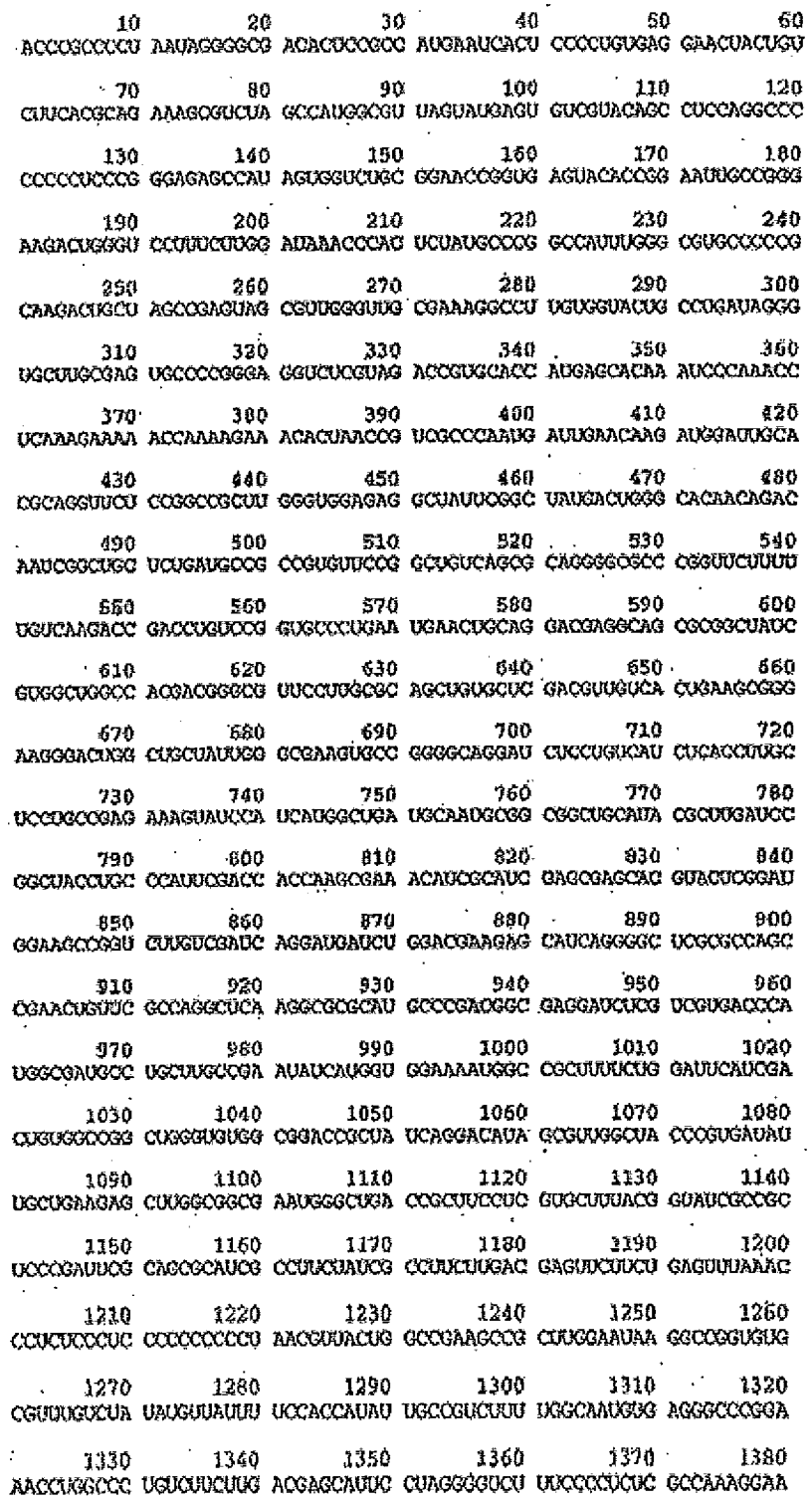
[Figure 3A]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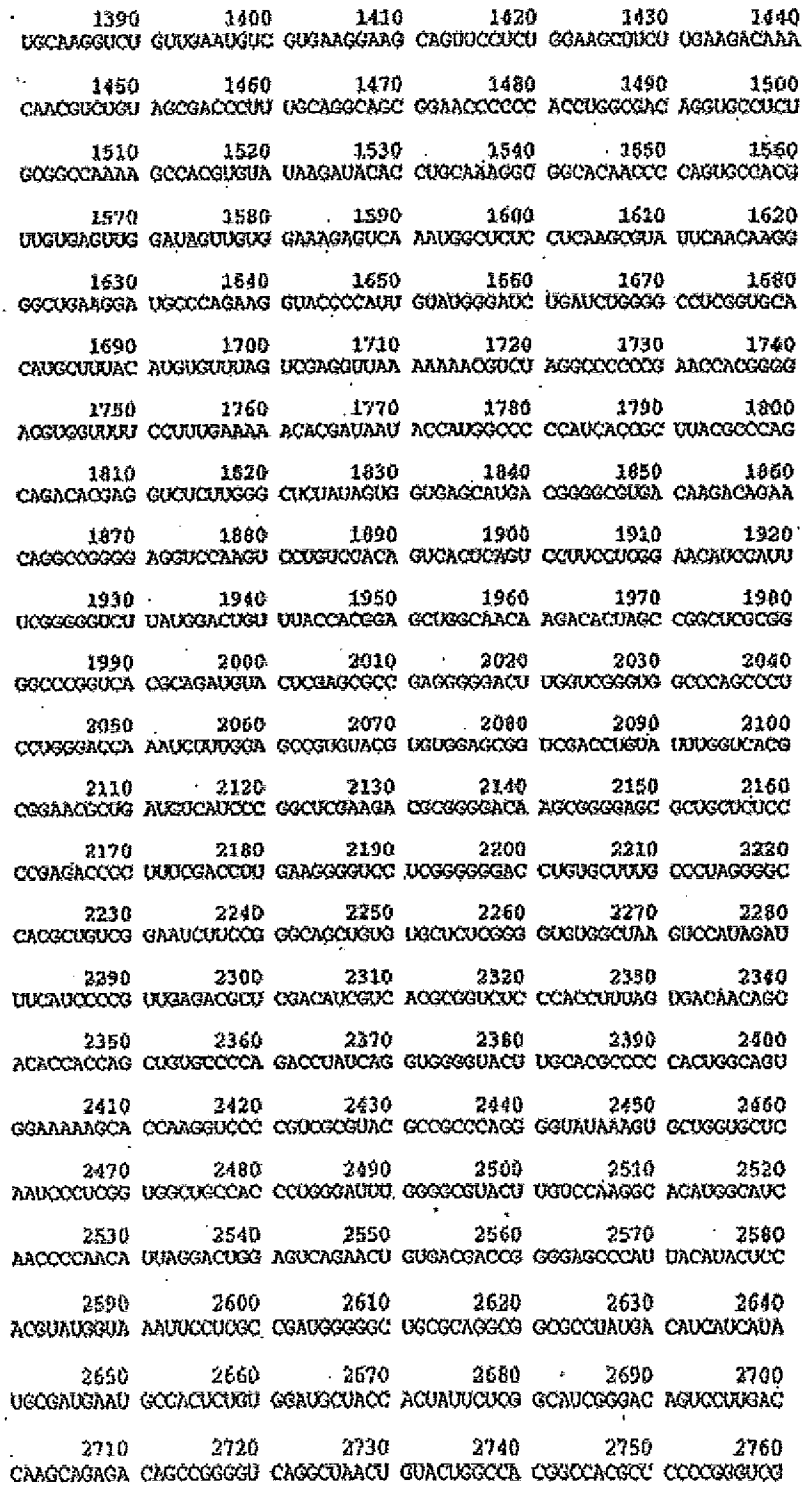
[Figure 3B]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1930 1940 1950 1960 1970 1980
UCGGGGGUCU UAUGGACUGU UUACCACGGA GCUGGCAACA AGACACUAGC CGGCUCGCGG
1990 2000 2010 2020 2030 2040
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2050 2060 2070 2080 2090 2100
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2110 2120 2130 2140 2150 2160
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2170 2180 2190 2200 2210 2220
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2230 2240 2250 2260 2270 2280
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2290 2300 2310 2320 2330 2340
UUCAUCCCCG UUGAGACGCU CGACAUCGUC ACGCGGUCUC CCACCUUUAG UGACAACAGC
2350 2360 2370 2380 2390 2400
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2410 2420 2430 2440 2450 2460
GGAAAAAGCA CCAAGGUCCC CGUCGCGUAC GCCGCCCAGG GGUAUAAAGU GCUGGUGCUC
2470 2480 2490 2500 2510 2520
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2530 2540 2550 2560 2570 2580
AACCCCAACA UUAGGACUGG AGUCAGAACU GUGACGACCG GGGAGCCCAU UACAUACUCC
2590 2600 2610 2620 2630 2640
ACGUAUGGUA AAUUCCUCGC CGAUGGGGGC UGCGCAGGCG GCGCCUAUGA CAUCAUCAUA
2650 2660 2670 2680 2690 2700
UGCGAUGAAU GCCACUCUGU GGAUGCUACC ACUAUUCUCG GCAUCGGGAC AGUCCUUGAC
2710 2720 2730 2740 2750 2760
CAAGCAGAGA CAGCCGGGGU CAGGCUAACU GUACUGGCCA CGGCCACGCC CCCCGUGUCG

[Figure 3C]

```
2770        2780        2790        2800        2810        2820
GUGACAACCC CCCAUCCCAA UAUAGAGGAG GUAGCCCUCG GACAGGAGGG UGAGAUCCCC

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2890        2900        2910        2920        2930        2940
CACUCAAAGA AAAAGUGUGA CGAGCUCGCA ACGGCCCUUC GGGGCAUGGG CUUGAACGCU

2950        2960        2970        2980        2990        3000
GUGGCAUAUU ACAGAGGGUU GGACGUCUCC AUAAUACCAA CUCAAGGAGA UGUGGUGGUC

3010        3020        3030        3040        3050        3060
GUUGCCACCG ACGCCCUCAU GACGGGGUAU ACUGGAGACU UUGACUCCGU GAUCGACUGC

3070        3080        3090        3100        3110        3120
AACGUAGCGG UCACCCAGGC CGUAGACUUC AGCCUGGACC CCACCUUCAC UAUAACCACA

3130        3140        3150        3160        3170        3180
CAGACUGUCC CGCAAGACGC UGUCUCAGGU AGUCAGCGCC GAGGGCGCAC GGGUAGAGGA

3190        3200        3210        3220        3230        3240
AGACUGGGCA UUUAUAGGUA UGGUUCCACU GGUGAGCGAG CCUCAGGAAU GUUUGACAGU

3250        3260        3270        3280        3290        3300
GUAGUACUCU GUGAGUGCUA CGACGCAGGA GCUGCUUGGU AUGAGCGCUC ACCAGUGGAG

3310        3320        3330        3340        3350        3360
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3370        3380        3390        3400        3410        3420
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3430        3440        3450        3460        3470        3480
CAGACAAAGC AGUCGGGGGA AAAUUUGCCA UACUUAGUAG CCUAUCAGGC CACAGUGUGC

3490        3500        3510        3520        3530        3540
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3550        3560        3570        3580        3590        3600
AAGCCCACGC UUUUGGGCCC UACACCCUCU CUGUACCGUU UGGGCUCUGU UACCAACGAG

3610        3620        3630        3640        3650        3660
GUCACCCUUA CACACCCCGU GACAAAAUAC AUCGCCACAU GCAUGCAAGC UGACCUCGAG

3670        3680        3690        3700        3710        3720
GUCAUGACCA GCACGUGGGU CCUGGCUGGG GGAGUCUUAG CAGCCGUCGC CGCGUAUUGC

3730        3740        3750        3760        3770        3780
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3790        3800        3810        3820        3830        3840
GCUCCGGACA AGGAGGUCCU CUAUGAGGCU UUUGAUGAGA UGGAGGAAUG UGCCUCCAGA

3850        3860        3870        3880        3890        3900
GCGGCUCUCC UUGAAGAGGG GCAGCGGAUA GCCGAGAUGC UGAAGUCCAA GAUCCAAGGC

3910        3920        3930        3940        3950        3960
UUAUUGCAGC AAGCCUCUAA ACAGGCCCAG GACAUACAAC CCGCUGUGCA AGCUUCGUGG

3970        3980        3990        4000        4010        4020
CCCAAGAUGG AGCAAUUCUG GGCCAAACAU AUGUGGAACU UCAUAAGCGG CAUUCAGUAC

4030        4040        4050        4060        4070        4080
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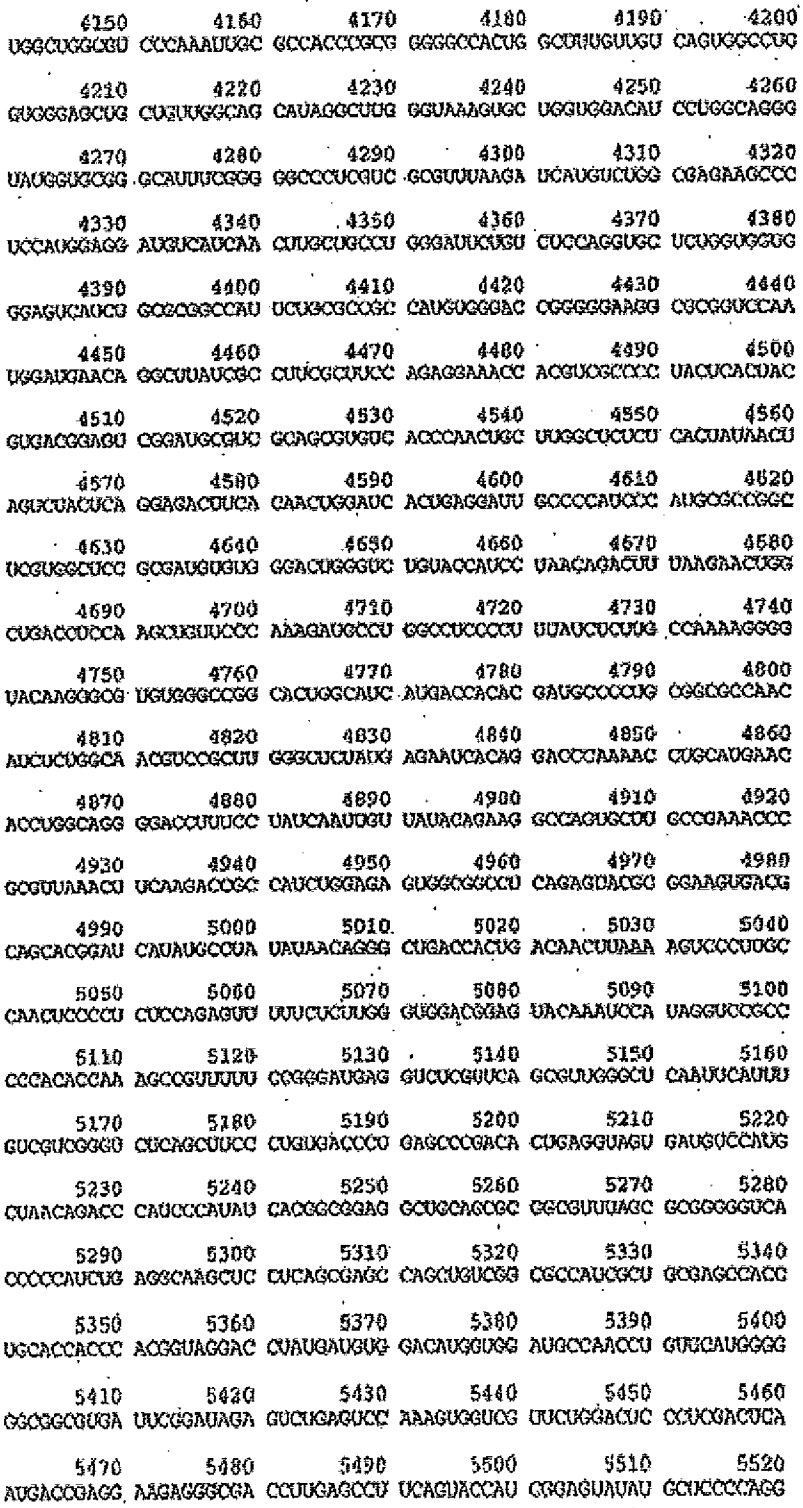
[Figure 3D]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4450 4460 4470 4480 4490 4500
4510 4520 4530 4540 4550 4560
4570 4580 4590 4600 4610 4620
4630 4640 4650 4660 4670 4680
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4750 4760 4770 4780 4790 4800
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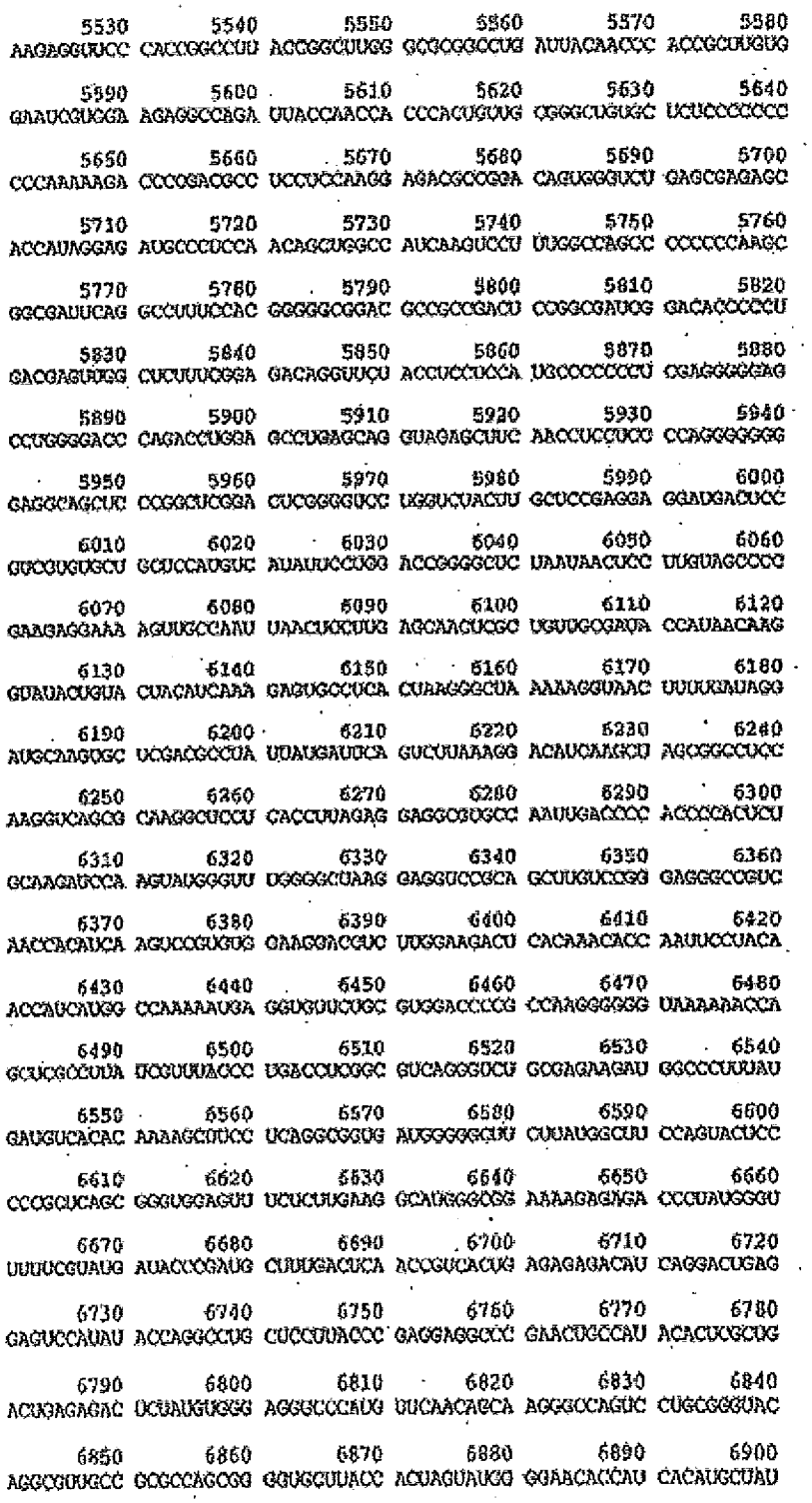
5410 5420 5430 5440 5450 5460
5470 5480 5490 5500 5510 5520

[Figure 3E]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5650 5660 5670 5680 5690 5700
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5710 5720 5730 5740 5750 5760
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5770 5780 5790 5800 5810 5820
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5830 5840 5850 5860 5870 5880
GACGAGUUGG CUCUUUCGGA GACAGGUUCU ACCUCCUCCA UGCCCCCCCU CGAGGGGGAG
5890 5900 5910 5920 5930 5940
CCUGGGGACC CAGACCUGGA GCCUGAGCAG GUAGAGCUUC AACCUCCUCC CCAGGGGGGG
5950 5960 5970 5980 5990 6000
GAGGCAGCUC CCGGCUCGGA CUCCGGGUCC UGGUCUACUU GCUCCGAGGA GGAUGACUCC
6010 6020 6030 6040 6050 6060
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6070 6080 6090 6100 6110 6120
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6130 6140 6150 6160 6170 6180
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6190 6200 6210 6220 6230 6240
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6250 6260 6270 6280 6290 6300
AAGGUCAGCG CAAGGCUCCU CACCUUAGAG GAGGCGUGCC AAUUGACCCC ACCCCACUCU
6310 6320 6330 6340 6350 6360
GCAAGAUCCA AGUAUGGGUU UGGGGCUAAG GAGGUCCGCA GCUUGUCCGG GAGGGCCGUC
6370 6380 6390 6400 6410 6420
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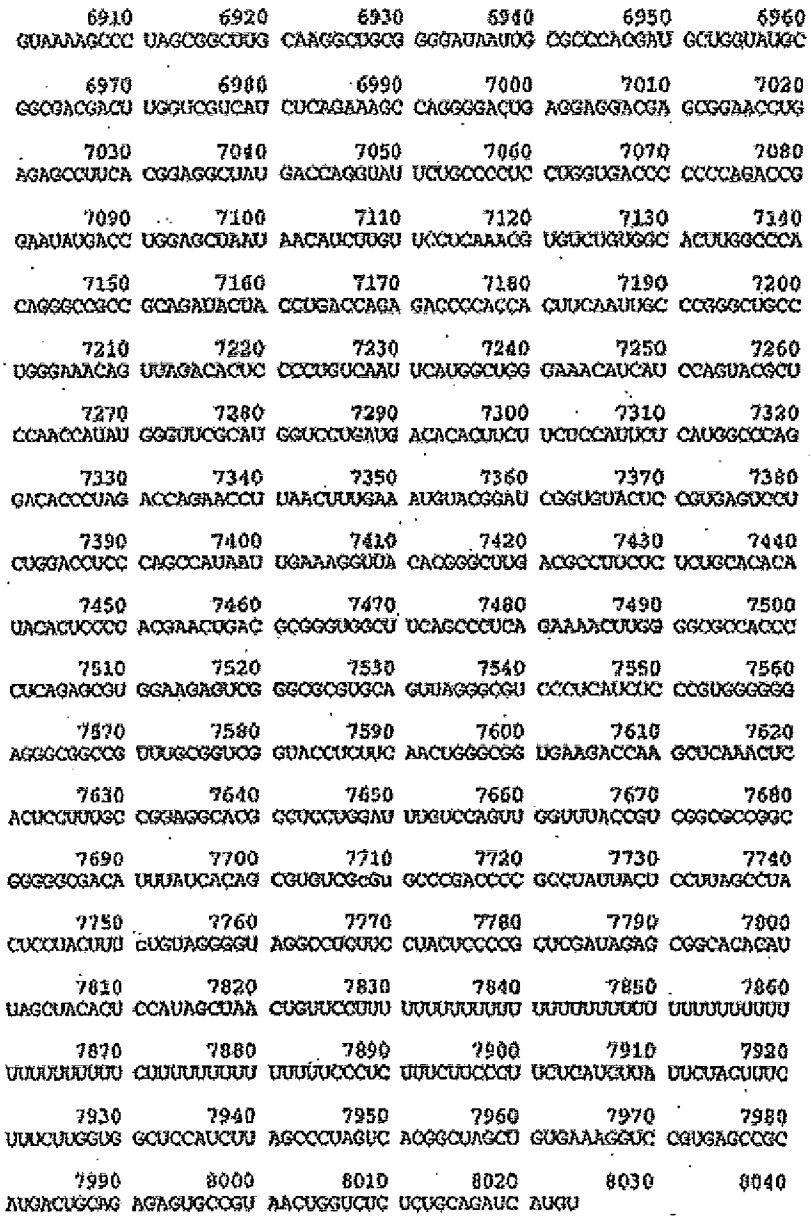
[Figure 3F]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7030 7040 7050 7060 7070 7080
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7090 7100 7110 7120 7130 7140
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7150 7160 7170 7180 7190 7200
CAGGGCCGCC GCAGAUACUA CCUGACCAGA GACCCCACCA CUUCAAUUGC CCGGGCUGCC
7210 7220 7230 7240 7250 7260
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7270 7280 7290 7300 7310 7320
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7330 7340 7350 7360 7370 7380
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7450 7460 7470 7480 7490 7500
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7870 7880 7890 7900 7910 7920
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7930 7940 7950 7960 7970 7980
UUUCUUGGUG GCUCCAUCUU AGCCCUAGUC AGGGCUAGCU GUGAAAGGUC CGUGAGCCGC
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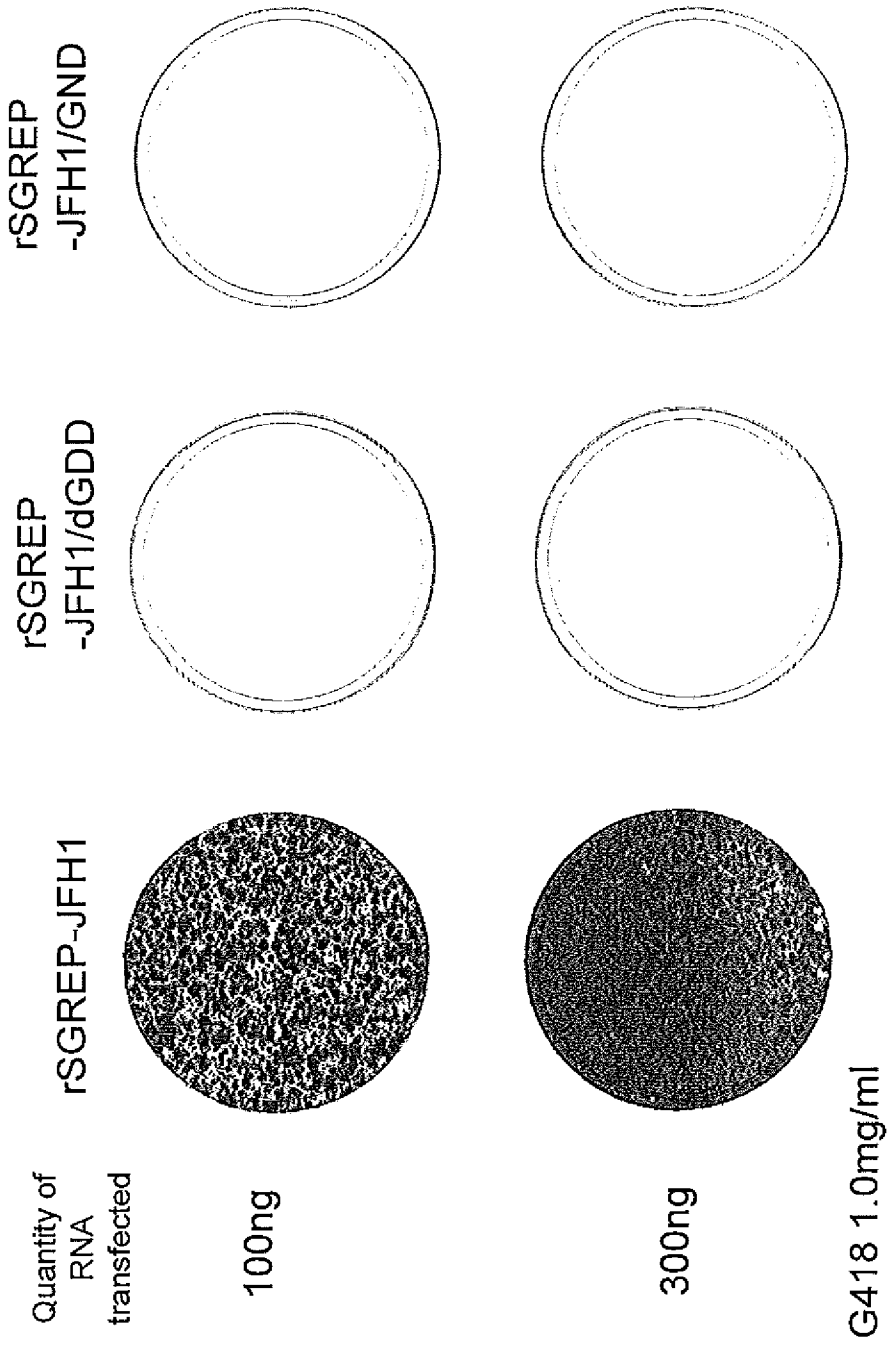
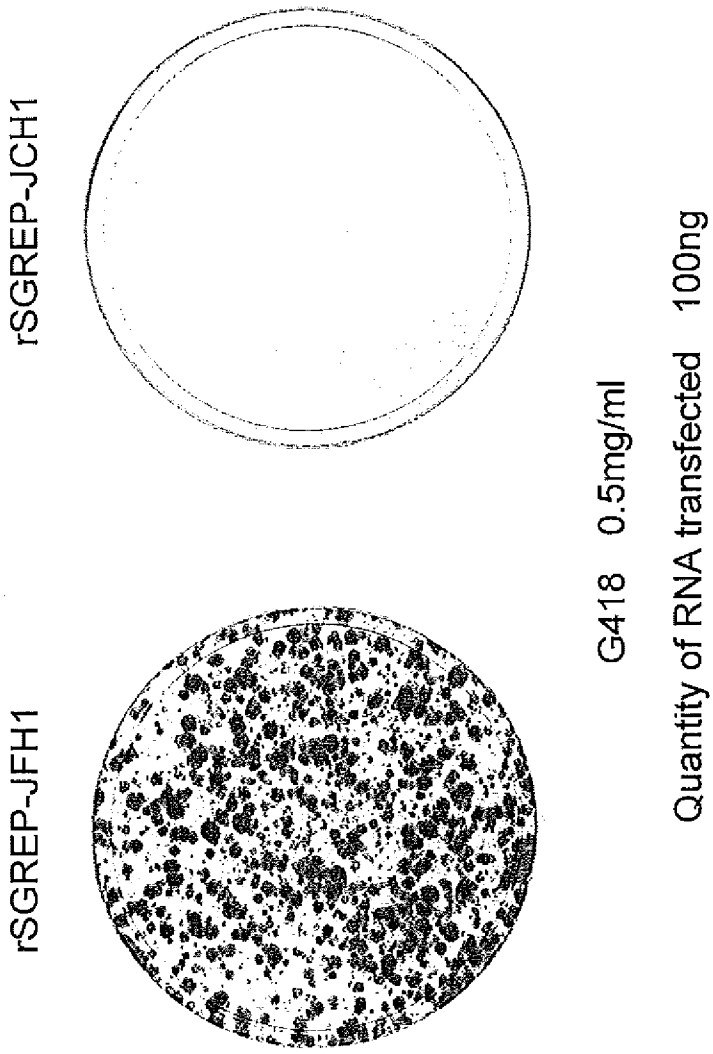
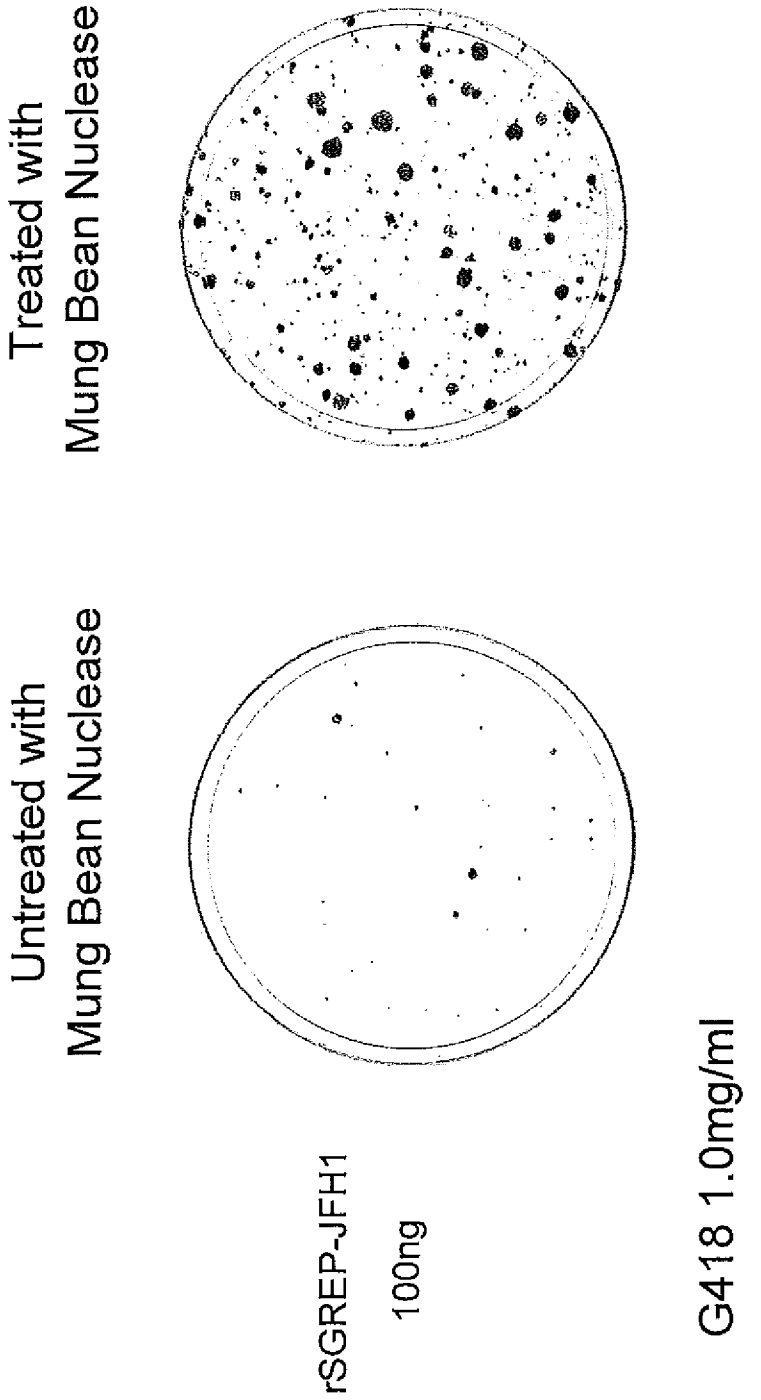
[Figure 4]


rSGREP
-JFH1/GND
rSGREP
-JFH1/dGDD
rSGREP-JFH1
Quantity of
RNA
transfected
100ng
300ng
G418 1.0mg/ml

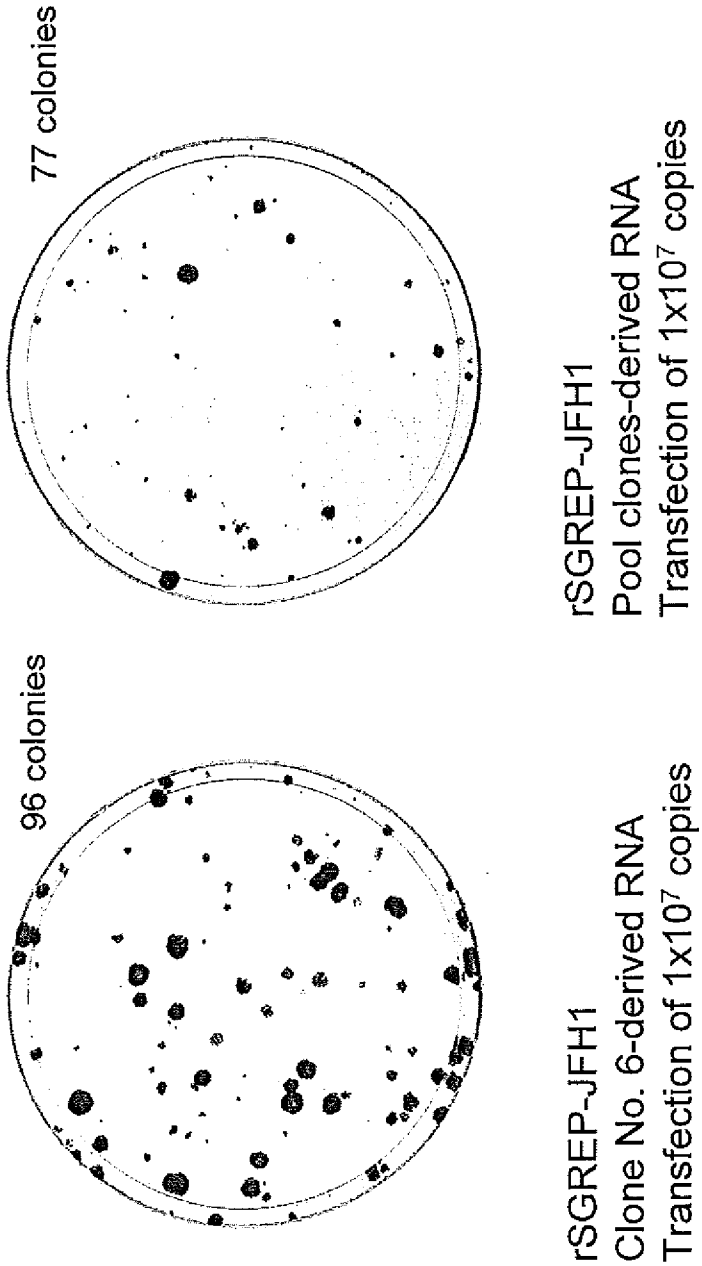
[Figure 5]


rSGREP-JCH1
rSGREP-JFH1
G418 0.5mg/ml
Quantity of RNA transfected 100ng

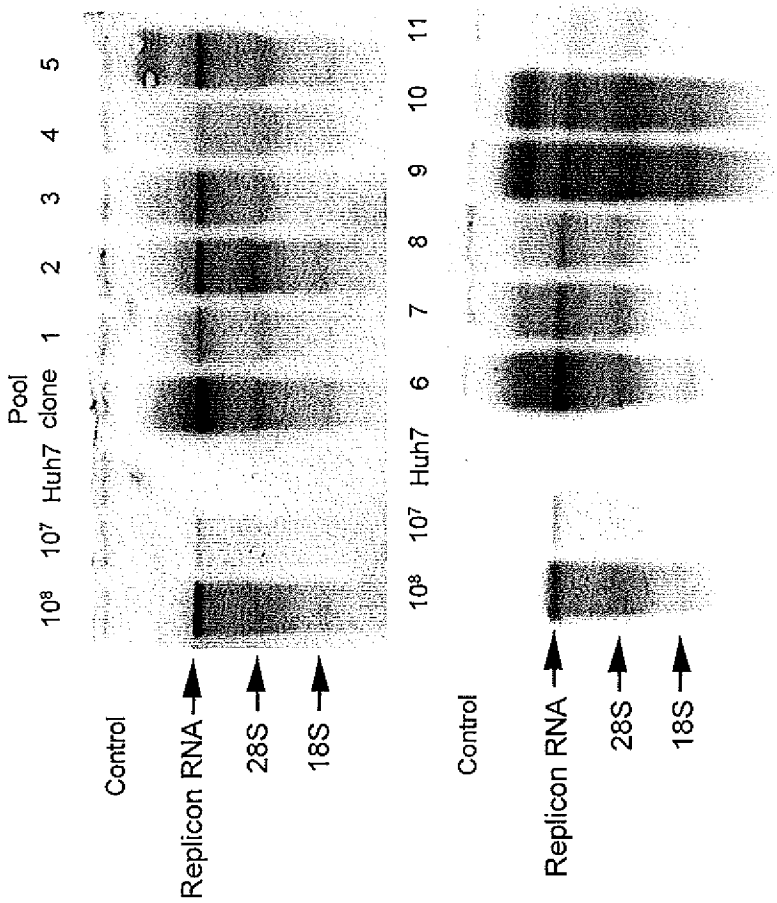
[Figure 6]


Treated with
Mung Bean Nuclease
Untreated with
Mung Bean Nuclease
rSGREP-JFH1
100ng
G418 1.0mg/ml

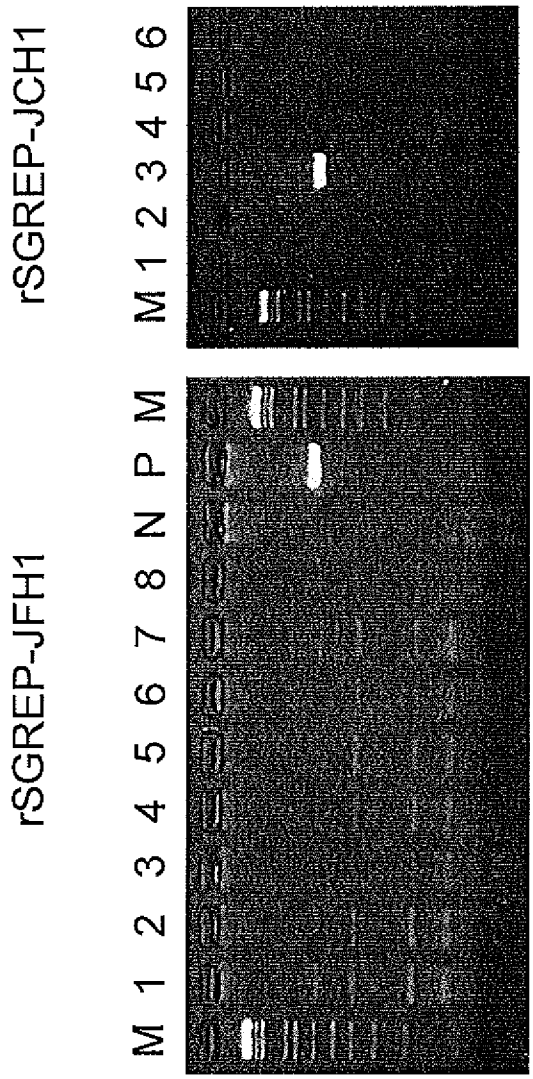
[Figure 7]


77 colonies
96 colonies
rSGREP-JFH1
Pool clones-derived RNA
Transfection of 1x10^7 copies
rSGREP-JFH1
Clone No. 6-derived RNA
Transfection of 1x10^7 copies

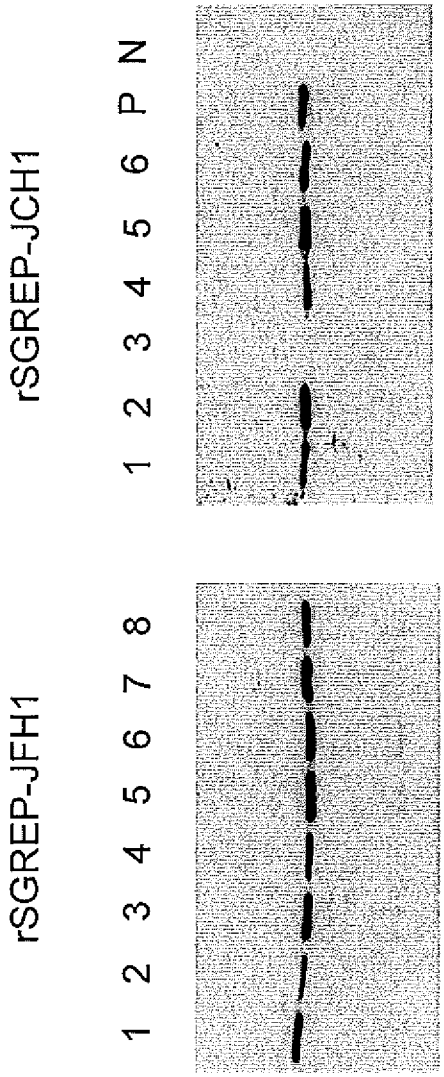
[Figure 8]


Pool
clone
Huh7
10⁸
10⁷
1
2
3
4
5
6
7
8
9
10
11
Control
Replicon RNA
28S
18S

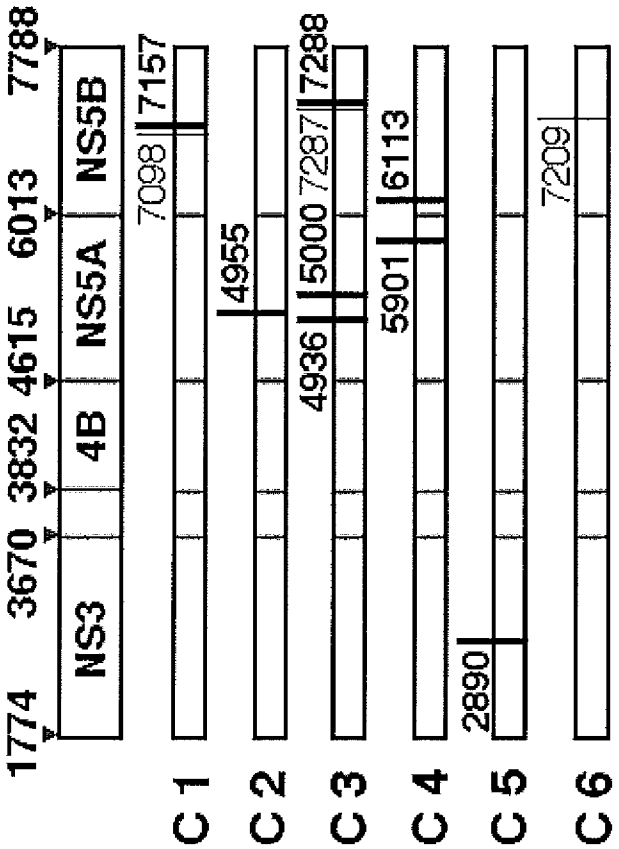
[Figure 9]



[Figure 10]



[Figure 11]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3832
4615
6013
7788
NS3
4B
NS5A
NS5B
7098
7157
4955
4936
5000
7287
7288
5901
6113
2890
7209
C 1
C 2
C 3
C 4
C 5
C 6

[Title of Document] ABSTRACT

[Abstract]

[Technical Problem] An object is to provide a replicon RNA that is derived from HCV of a different genotype from genotype 1b.

[Technical Solution] A replicon RNA comprising a nucleotide sequence at least containing the 5' untranslated region, the sequence encoding NS3 protein, NS4A protein, NS4B protein, NS5A protein and NS5B protein, and the 3' untranslated region on the genomic RNA of hepatitis C virus of genotype 2a is provided.

[Selected Drawing] None